THE AMERICAN REVIEW OF RESPIRATORY DISEASES

OFFICIAL JOURNAL OF THE AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF THE NATIONAL TUBERCULOSIS ASSOCIATION

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VOLUME 80
JULY-DECEMBER, 1959

PUBLISHED MONTHLY
AT MT. ROYAL AND GUILFORD AVENUES, BALTIMORE 2, MD.
BY THE NATIONAL TUBERCULOSIS ASSOCIATION

CONTENTS: ORIGINAL ARTICLES

NUMBER 1 (PART 1), JULY, 1959

Studies on the Gaseous Content of Tuberculosis Cavities. JAAKKO H. HAAPANEN, IRVING KASS, GOFFREDO GENSINI, AND GARDNER MIDDLEBROOK 1

Analysis and Late Results of Resection for Pulmonary Tuberculosis in Hawaii. PAUL W. GEBAUER AND CARL B. MASON 6

A Factor in Serum Which Prevents Propagation of D-29 Mycobacteriophage with Human Tubercle Bacilli. ARTHUR WHITE AND VERNON KNIGHT 12

The *In Vitro* Effect of Specific and Nonspecific Bacterial Products on the Migration of Leukocytes from Tuberculin-sensitized Guinea Pigs. J. WASSERMAN AND TH. PACKALÉN 19

The Inhibition of Isoniazid Inactivation by Means of PAS and Benzoyl-PAS in Man. H. LAUENER AND G. FAVEZ 26

Mechanics of Breathing. I. The Physical Properties of the Lung. ERNST O. ATTINGER AND MAURICE S. SEGAL 38

Mechanics of Breathing. II. The Respiratory Work. ERNST O. ATTINGER AND MAURICE S. SEGAL 46

Mechanics of Breathing. III. The Influence of Ventilatory Mechanics upon Gas Exchange and Pulmonary Circulation. ERNST O. ATTINGER AND MAURICE S. SEGAL. 53

Is a Vocational Rehabilitation Program Justified in a Tuberculosis Hospital? ARTHUR J. MARION AND DAVID SALKIN 59

Case Reports:

Endobronchial Hamartoma. ROBERT A. GREEN 65

Evolution of Pulmonary Sarcoidosis as Revealed in a Case Followed Over a Six-Year Period. J. ROBERT THOMPSON AND HANS POPPER 71

Silicosis and Avian Tuberculosis. RICHARD P. JAHN 78

Notes:

Effectiveness of Antituberculous Treatments Tested by Direct Culture of the Bacilli in the Patient's Blood. B. KREIS 85

Cycloserine-Isoniazid in the Ambulatory Treatment of Active Tuberculosis After Failure of Previous Chemotherapy. V. LEITES 89

The "Bluing" Phenomenon: A Source of Contamination in Cultures of Tubercle Bacilli. D. GALE AND E. A. LOCKHART 95

Editorial:

The Natural History of Tuberculosis in the Human Body. II. Longitudinal Observations Imperative. J. ARTHUR MYERS 100

Correspondence:

Chemotherapy to Prevent Relapse in Patients with Noninfectious Tuberculosis. W. H. OATWAY, JR.; **Further Observations on the Correlation Between Isoniazid Serum Concentrations and Therapeutic Response in Human Pulmonary Tuberculosis.** R. S. MITCHELL, *et al.*; **Tuberculosis was Fashionable in 1759.** C. J. ROBB; **Differential Uptake of Isoniazid-C^{14} by *Mycobacterium Paratuber-***

culosis Susceptible and Resistant to Isoniazid and Hydrogen Peroxide. S. Garttini *et al.*; Rehabilitation of Tuberculosis Patients. S. H. Dressler and M. Nacman 108

Books 113

American Trudeau Society:

The Chest Roentgenogram and Chest Roentgenographic Surveys Related to X-Ray Radiation Effects and Protection from Radiation Exposure. A Statement of the Committee on Radiation Effects 115

The "Open-Negative" Problem. A Statement of the Committee on Therapy .. 118

Obituaries 120

Necrology 122

Notices 123

Abstracts 125

Number 1 (Part 2), July, 1959

Symposium on Emphysema and the "Chronic Bronchitis" Syndrome 1–213

Number 2, August, 1959

An Evaluation of the Specificity and Sensitivity of a Gel Double-Diffusion Test for Tuberculosis. A Double-blind Study. Robert C. Parlett and Guy P. Youmans .. 153

Clinical and Immunologic Significance of *Aspergillus Fumigatus* in the Sputum. J. Pepys, R. W. Riddell, K. M. Citron, Y. M. Clayton, and E. I. Short 167

Clinical and Physiologic Results following Radioactive Iodine (I^{131}) in the Treatment of Chronic Pulmonary Insufficiency. Lois T. Ellison, B. Shannon Gallaher, Irene F. LaMotte, William F. Hamilton, Jr., Robert G. Ellison, and W. F. Hamilton 181

Chronic Pulmonary Disease due to Atypical Mycobacterial Infections. Albert G. Lewis, Jr., Frank P. Dunbar, Eunice M. Lasche, James O. Bond, Ernest N. Lirner, Dwight J. Wharton, Albert V. Hardy, and Roberts Davies 188

Tuberculosis Among the Navajo. Research in Cross-cultural Technologic Development in Health. Kurt Deuschle 200

Surgery in Pulmonary Tuberculosis. A Review. Roger S. Mitchell and Oscar Auerbach 207

Some Fractions of Tubercle Bacillus Wax and Their Immunogenicity for Mice. Anson Hoyt, Millie A. Thompson, Frederick J. Moore, and C. Richard Smith 216

Nutritional Studies of Isoniazid in Rats Deficient in Vitamin E. T. Moore and I. M. Sharman 223

A Temperate Bacteriophage from *Mycobacterium Butyricum*. B. U. Bowman, Jr., and W. B. Redmond 232

The Mechanism of the Toxicity of Cord Factor. Masariko Kato, Masamichi Kusunose, Katsuji Miki, Kiyoteru Matsunaga, and Yuichi Yamamura 240

Case Report:

Pulmonary Alveolar Proteinosis. A Case Report with Unusual Clinical and Laboratory Manifestations. Francis B. Landis, Harold D. Rose, and Richard O. Sternlieb 249

Editorial:

Standardization and Stability of Purified Tuberculin. J. GULD 255

Notes:

Studies of the Catalase Activity of *Mycobacterium Tuberculosis* Strain H37Rv. W. TYSAROWSKI AND S. KWIEK 257
Tuberculous Polyserositis. C. G. SPIVEY AND J. A. WIER 259
Instability of Penicillin in Dubos Media. E. M. K. VAICHULIS AND J. P. DOLL 262
***Histoplasma Capsulatum* and *Blastomyces Dermatitidis* Polysaccharide Skin Tests on Humans.** R. A. KNIGHT, S. CORAY, AND S. MARCUS 264
A New Liquid Synthetic Medium for the Cultivation of Mycobacterium Species. C. ALDRIDGE *et al.* 267
Evaluation of Cycloserine with High Dosage of Isoniazid in Chronic Treatment-Failure Pulmonary Tuberculosis. E. WOLINSKY AND V. N. KAPUR 269
Further Observations on the Biologic Activity of Crude Mycobacterial Cells. G. L. HOBBY *et al.* 274

Correspondence:

Source of Scotochromogens. E. H. RUNYON; **Historic Collection of Pneumothorax Machines and Needles.** T. MARMION 277
Books 279

American Trudeau Society: Notices 282

Abstracts 283

NUMBER 3, SEPTEMBER, 1959

The J. Burns Amberson Lecture. Viral Infections of the Respiratory Tract. FRANK L. HORSFALL, JR. 315
Infection of Children by Strains of Tubercle Bacilli Initially Resistant to Streptomycin or to Isoniazid. ROBERT DEBRÉ, H. NOUFFLARD, H. E. BRISSAUD, AND J. GERBEAUX 326
Vaccination against Tuberculosis with Nonliving Vaccines. I. The Problem and Its Historical Background. DAVID W. WEISS 340
Tuberculosis in Taiwan (Formosa). J. C. T'AO 359
Hepatic Toxicity of Pyrazinamide Used with Isoniazid in Tuberculous Patients. A UNITED STATES PUBLIC HEALTH SERVICE TUBERCULOSIS THERAPY TRIAL 371
Tuberculous Meningitis. Prognosis and Treatment. B. F. VOLJAVEC, S. P. ORTON, AND R. F. CORPE 388
Passive Transfer of a Delayed Type of Skin Reactivity to Tuberculin PPD. LEON R. COLE, RITA L. PALDINO, RUTH W. HENDERSON, AND ANN F. WEST 398
The Effect of Isoniazid on the Synthesis of Certain Amino Acids and Vitamins by *Mycobacterium Tuberculosis*. HILDA POPE WILLETT 404
Specific and Nonspecific Inhibition of Epithelial Growth in Tissue Cultures of Kidney Explants from Tuberculin-sensitized Guinea Pigs. TH. PACKALÉN, S. TUNÇMAN, AND J. WASSERMAN 410

Notes:

The Use of Riboflavin as an Indicator of Isoniazid Ingestion in Self-medicated Patients. G. L. Hobby and K. W. Deuschle 415
Streptovaricin and Isoniazid in the Treatment of Pulmonary Tuberculosis. A. Nathan 424
Streptovaricin Alone in the Treatment of Active Pulmonary Tuberculosis. A Short-Term Clinical Study. E. A. Riley *et al.* 426
Streptovaricin and Isoniazid in the Treatment of Pulmonary Tuberculosis. R. Des Prez *et al.* 431
Atypical Acid-fast Bacilli. III. An Expanded Schema. S. McMillen and D. S. Kushner 434
Fibrin-Clot Culture Technique for Isolation of Tubercle Bacilli from Pleural Exudates. L. Sula 438
The Innocuousness and Possible Therapeutic Use of Aerosol Amphotericin B. K. H. Kilburn 441
A Factor in Serum Albumin Which Prevents the Inhibition of Propagation of D-29 Mycobacteriophage by Tween. A. C. White and V. Knight 443

Correspondence:

Pancreas versus Omentum in Experimental Tuberculosis. C. E. Woodruff; Rehabilitation and Occupational Therapy in Tuberculosis Hospitals. F. C. Warring, Jr.; A. R. Allen; Terminology Used for Discharges from Hospital, D. Salkin and Editor 445
Books 450

American Trudeau Society:

Obituaries 452
Notices 455

Abstracts 457

Number 4 (Part 1), October, 1959

Chronic Bronchitis. Its Prevalence, Nature, and Pathogenesis. C. M. Fletcher 483
Vaccination Against Tuberculosis with Nonliving Vaccines. I. The Problem and Its Historical Background (*continued*). David W. Weiss 495
Some Effects of Mechanical Respirators upon Respiratory Gas Exchange and Ventilation in Chronic Pulmonary Emphysema. A. Gregory Jameson, M. Irené Ferrer, and Rejane M. Harvey 510
Chemotherapy and Pathology of Experimental Photochromogenic Mycobacterial Infections. Emanuel Wolinsky 522
Biochemical Analysis of the Virulence of Tubercle Bacilli. Masahiko Kato, Katsuji Miki, Kiyoteru Matsunaga, and Yuichi Yamamura 535
Studies on the Biologic Properties of Mycobacteriophage. Kenji Takeya, Tetsuya Yoshimura, Kenji Yamaura, and Tadao Toda 543
Reinfection in Guinea Pigs with Tuberculosis Arrested by Chemotherapy. J. Cerbón S., L. F. Bojalil, and Ruy Pérez-Tamayo 554
Amithiozone: Mechanism of Action and Resistance Development by Mycobacteria. M. O. Tirunarayanan, W. A. Vischer, and H. Bruhin 559

The Tuberculin Reaction of 530 Patients Admitted to the Tuberculosis Service, Fitzsimons Army Hospital. JAMES A. WIER AND JAMES M. SCHLESS 569

Case Reports:

Idiopathic Hypoventilation, Polycythemia, and Cor Pulmonale. L. THEODORE LAWRENCE 575

Editorial:

Air Pollution and Bronchitis. ALBERT ROBERTS 582

Notes:

A Simple Paper Strip Urine Test for Para-Aminosalicylic Acid. A. D. CHAVES 585

Effect of Topical Hydrocortisone Acetate Ointment on the Tuberculin Skin Reaction. D. LEVY *et al.* 587

A Clinical Evaluation of Thiocarbanidin and Isoniazid in the Treatment of Pulmonary Tuberculosis. R. F. DONOHOE *et al.* 590

Books 594

American Trudeau Society: Notices 597

Abstracts 599

NUMBER 4 (PART 2), OCTOBER, 1959

International Symposium of the Deborah Sanatorium and Hospital 1–139

NUMBER 5, NOVEMBER, 1959

Sequential Use of Paired Combinations of Isoniazid, Streptomycin, Para-Aminosalicylic Acid, and Pyrazinamide. A UNITED STATES PUBLIC HEALTH SERVICE TUBERCULOSIS THERAPY TRIAL 627

VII. Comparison of Isoniazid Alone with Isoniazid—PAS in the Original Chemotherapy of Noncavitary Pulmonary Tuberculosis. Results from the Veterans Administration—Armed Forces Co-operative Study on the Chemotherapy of Tuberculosis. SAMUEL PHILLIPS 641

The Chemoprophylaxis of Tuberculosis. A Review. H. P. LAMBERT 648

Fatality Ratio for Lupus Vulgaris Cutis. OLE HORWITZ 659

Vaccination against Tuberculosis with Nonliving Vaccines. I. The Problem and Its Historical Background (*concluded*). DAVID W. WEISS 676

Cardiopulmonary Function in Chronic Obstructive Emphysema. M. HENRY WILLIAMS, JR. AND LENORE R. ZOHMAN 689

Cardiopulmonary Function in Pulmonary Fibrosis. LENORE R. ZOHMAN AND M. HENRY WILLIAMS, JR. 700

The Diagnosis of Emphysema: A Physical Sign, a Roentgenographic Sign, and an Oximeter Test. C. R. WOOLF 705

Chemoprophylaxis in Chronic Obstructive Pulmonary Emphysema. A Twelve-Week Study with Erythromycin. WILBUR Y. HALLETT, GILDON N. BEALL, AND WILLIAM M. M. KIRBY 716

The Expiratory Ventilagram. Application of Total and Timed Vital Capacities and Maximal Expiratory Flow Rate, as Obtained by a Bellows Apparatus, for Bedside and Office Use. GLENN E. HORTON AND SAMUEL PHILLIPS 724

Case Reports:

Rheumatoid Lung Disease. Report of a Case Which Developed in Childhood. Geoffrey L. Brinkman and Leo Chaikof 732
Pulmonary Disease Due to Atypical Tubercle Bacilli. Frederick Beck 738

Notes:

Growth Characteristics of Acid-fast Microorganisms other than Tubercle Bacilli in HeLa Cells. M. E. Clark and E. Forrest 744
Isolation of "Atypical" Mycobacteria from Healthy Persons. L. B. Edwards and C. E. Palmer 747
The Relationship of Sex to the Susceptibility of Normal and Immunized Mice to Tuberculosis. G. P. Youmans and A. S. Youmans 750
The Difference in Response of Four Strains of Mice to Immunization against Tuberculous Infection. G. P. Youmans *et al.* 753
Controlled Clinical Trial of Streptovaricin—Isoniazid. A U. S. Public Health Service Tuberculosis Therapy Trial 757

Correspondence:

Failure to Develop Sarcoidosis after the Oral Ingestion of Pine Pollen. J. H. Richert 760
Books 761

American Trudeau Society: Notices 764

Abstracts 765

Number 6, December, 1959

Prevalence of Tuberculosis among Selective Service Registrants Currently Processed for Military Service. Bernard D. Karpinos 795
Measurements of the Pulmonary Diffusing Capacity for Oxygen during Exercise. Arthur A. Siebens, N. Robert Frank, Donald C. Kent, Melvin M. Newman, Robert A. Rauf, and Betty L. Vestal 806
Objective Long-Term Evaluation of the Surgical Treatment of Diffuse Obstructive Emphysema. A Physiologic Study. Burton S. Tabakin, Prasant K. Adhikari, and Donald B. Miller 825
Pulmonary Involvement in Leukemia. Robert A. Green and Norman J. Nichols 833
Studies of Thyroid Function in Patients Treated with Para-Aminosalicylic Acid and Isoniazid. Edward Seinfeld and Paul Starr 845
Virulence of the Tubercle Bacillus. III. Effect of Penicillin upon the Growth of Virulent and Avirulent Bacilli. Charles T. Uyeda and Sidney Raffel 849
The Detection of Pyrazinamide-induced Liver Damage by Serum Enzyme Determinations. John F. Morrissey and Ruth C. Rubin 855
Desoxyribonucleic Acid as Growth Stimulant of Tubercle Bacilli. E. Darzins 866
Antituberculous Activity of a Phenazine Derivative (B663). B. K. Acharya, J. M. Robson, and F. M. Sullivan 871
A Comparison of the Virulence of Various Known and Atypical Mycobacteria for Chickens, Guinea Pigs, Hamsters, and Mice. Frederick E. Durr, Donald W. Smith, and David P. Altman 876

Further Studies on Gel Diffusion Tests in Tuberculosis. I. Methods for Standardization of the Antigen and Detection of Small Amounts of Antibody. ROBERT C. PARLETT AND CAROLYN A. REHR 886

Case Report:

Alveolar-Capillary Block Due to Leukemic Infiltration of the Lung. ROBERT A. GREEN, NORMAN J. NICHOLS, AND EDWARD J. KING 895

Notes:

The Predicted Maximal Breathing Capacity in Obese Subjects. G. C. LEINER AND S. ABRAMOWITZ 902

Comparative Study of Urine Tests for the Detection of Isoniazid. R. W. ROBERTS AND K. W. DEUSCHLE 904

Nasal Swab Cultures in Patients with Pulmonary Tuberculosis. R. D. TREVATHAN AND S. PHILLIPS 909

Failure of Desoxyribonucleic Acid from Mycobacteria to Induce Bacterial Transformation. H. BLOCH *et al.* 911

Some Observations on the Use of Benzalkonium Chloride (Zephiran®) in Tuberculosis Bacteriology. L. G. WAYNE 912

Homogenization of Viscous Sputum. A New Method. J. H. ALLI AND T. E. CORCORAN 914

Longitudinal Studies in Emphysema. III. Sputum Eosinophilia. M. B. COLE *et al.* 915

Books 919

American Trudeau Society:

Obituaries 921

Notices 924

Index of Authors 925

Index of Subjects 928

Index of Abstracts 937

For more than a decade, repeated consideration has been given to the question of modifying the name of the REVIEW in order to portray more faithfully its actual content. On the one hand was the natural desire to maintain "tuberculosis" in the title. On the other hand, it seemed only proper to show recognition of the REVIEW's steadily increasing coverage of studies in the field of nontuberculous diseases of the respiratory tract such as those presented in the supplement that forms a part of this issue.

The addition "AND PULMONARY DISEASES" in 1955 was an attempt to make the title more accurate, but it also made it cumbersome. Accordingly, it has been decided that the time has come to shorten the title without sacrificing its descriptive quality and, with this issue, the title becomes THE AMERICAN REVIEW OF RESPIRATORY DISEASES.

It is our intention to continue in the future as we have in the past to devote full coverage to studies of every aspect of tuberculosis and the other mycobacterial diseases, while at the same time publishing significant work from the clinic and the laboratory concerned with all other diseases of the respiratory tract.

The Editor

Letter from the Editor

The Editor wishes to take this opportunity to express his appreciation to Dr. William E. Adams, Dr. Max B. Lurie, Dr. Gardner Middlebrook, and Dr. George W. Wright for their high quality of performance and diligent service as members of the Editorial Board.

New members of the Board as of July 1, 1959, are Dr. Oscar Auerbach, Dr. Lyman A. Brewer, III, Dr. Julius Comroe, Jr., and Dr. Sidney Raffel, who have accepted three-year appointments.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

VOLUME 80 *July 1959* NUMBER 1, PART 1 OF TWO PARTS

STUDIES ON THE GASEOUS CONTENT OF TUBERCULOUS CAVITIES[1]

JAAKKO H. HAAPANEN, IRVING KASS, GOFFREDO GENSINI, AND GARDNER MIDDLEBROOK

(Received for publication October 10, 1958)

INTRODUCTION

Following Roentgen's discovery, direct examination of the tuberculous cavity became technically possible. Ameuille (1) was the first to outline, with the direct injection of contrast medium, the contour and the communicating bronchi of an intrapulmonary cavity. In 1930, Coryllos and his co-workers studied the genesis of atelectasis and the healing of tuberculous cavities by artificial bronchial obstruction (2). They also measured the intracavitary pressure and analyzed the gaseous content of four tuberculous cavities (3, 4). Eloesser (5–7) also made extensive studies by direct needling of tuberculous cavities.

The purpose of this preliminary report is to show the variations of the gaseous content of the tuberculous cavity, especially in relation to whether the communicating bronchi are open or closed.

MATERIALS AND METHODS

Twenty patients with chronic pulmonary tuberculous cavities and receiving antimycobacterial treatment were studied.

Before the study the patient was shown his chest roentgenograms, and it was made clear that the knowledge of the bacteriologic status of the cavity as well as the degree of patency of the communicating bronchi, as judged by changes in the intracavitary pressure and by the fate of the radiopaque material injected into the cavity, might be useful in the future management of the disease. Patients were also told that some steps, e.g., the air analysis, were done mainly for investigational purposes. In addition, the possible complications of this procedure were discussed with the patient. It was explained that slight bleeding into the cavity was probable and that some blood-streaking would, therefore, be encountered. The possibility of a partial pneumothorax was also made clear. Finally, the patient was informed that during the procedure he would be told to refrain from coughing until the roentgenograms were taken. After this intensive explanation all of these 20 patients volunteered for the study.

The 20 cavities measured between 1.5 by 4 cm. and 6 by 15 cm. on routine chest roentgenograms. They were located in the upper lobes, 9 on the right and 11 on the left. One case was eliminated because the cavity was so small that it did not contain much more air than the amount initially removed for examination. For the present studies, a modification of the previously described speleography[2] technique was used (8).

Transparietal needling of the cavity: Premedication consisted of 30 mg. of hypodermic codeine, given one-half hour before the procedure. In a few cases, pentobarbital sodium (50 mg.) was necessary to relieve the anxiety.

The site of needling, usually the anterior chest wall, was determined by fluoroscopy. The skin and subcutaneous tissues were infiltrated with procaine or lidocaine (Xylocaine®). A 16-gauge needle 4 to 6 inches in length with an attached

[1] From the departments of Medicine, and Research and Laboratories, National Jewish Hospital at Denver; and the departments of Medicine and Microbiology, University of Colorado School of Medicine, Denver, Colorado.

[2] The word "speleology" was proposed to be used for the collective information on the pathogenesis, physiology, morphology, bacteriology, and evolution of pulmonary cavities obtained by roentgenographic methods, pathologic examination of resected or autopsy specimens, and by direct intracavitary studies on living subjects. The word is derived from the Greek words "spelaion" (cave, cavity) and "logos" (word, thought, reason). Thus the word "speleology" literally means the science of exploring cavities or caves. The word "speleography" ("graphein," to engrave, write) has been used for the visualization of cavities by direct injection of radiopaque material.

oiled stopcock was passed through the chest wall. When the needle had passed the parietal pleura, manometric pressures were recorded with a P23g Statham strain gauge and a Sanborn 150 recording apparatus. If there was a free pleural space, the procedure was stopped because of the risk of traumatic pneumothorax and/or tuberculous empyema. If no pleural pressure was observed, the needle, under fluoroscopic control, was pushed slowly forward until it reached the cavity. Entrance into the cavity was often recognized by feeling a sudden decrease in resistance to the advancing needle.

To verify that the tip of the needle was in the cavity, as well as to obtain information about the degree of patency of the communicating bronchi whenever possible, the following procedures were performed:

1. The initial intracavitary gas pressure was recorded.
2. An air sample (10 to 20 cc.) was drawn into an oiled syringe through an oiled stopcock which was then closed. The carbon dioxide and oxygen content were determined according to the Scholander microvolumetric technique (9).
3. More air was drawn from the cavity.
4. Another air sample was taken as described above.
5. The second intracavitary gas pressure was measured.
6. Intracavitary fluid was aspirated for bacteriologic examination; in the absence of fluid, saline lavage was performed.
7. Finally, propyliodine (oily Dionosil®) was injected into the cavity, and the movement of the contrast medium was followed by serial roentgenograms, usually with fluoroscopic examinations.

Complications: In spite of precautions, pneumothorax was discovered in 4 of the 20 patients following completion of the procedure. In 2 of these patients, the air had to be aspirated before the lung was completely expanded. A slightly blood-tinged sputum was often noticed for a few days after completion of the procedure. If bleeding into the cavity was noted during the procedure, no lavage was done, nor was any propyliodine introduced.

TABLE 1

GAS ANALYSIS RESULTS

Case	Initial Air Sample		Amount of Gas Removed Between Studies	Repeat Air Sample	
	CO_2 Vols. Per Cent	O_2 Vols. Per Cent	cc.	CO_2 Vols. Per Cent	O_2 Vols. Per Cent
Group I: Open Cavities: Pressure Zero					
R.I. (left)	1.1	19.8			
W.D.	2.0	18.7	50	1.7	18.8
E.F.	2.5	18.5	50	2.6	17.9
O.G.	4.2	16.6	300	3.6	17.0
J.G.	4.3	19.2	20	4.3	19.2
M.M.	5.1	17.5			
R.B.	5.2	14.2	200	3.4	16.8
Average	3.5	17.8			
Group II: Partially Blocked Cavities: Pressure Negative					
R.I. (right)	3.3	18.7			
F.K.	3.6	15.6			
A.L.	3.7	16.2	300	8.9	16.0
A.L. (repeat)	3.2	17.3	20	3.3	17.8
H.M.	4.9	14.7			
L.T.	5.1	11.6			
L.P.	6.5	10.5	200	6.5	10.9
J.R.	7.2	7.7			
Average	4.7	13.6			
Group III: Partially Blocked Cavity: Pressure Positive (Tension Cavity)					
A.E.	3.1	18.9			
Group IV: Blocked Cavities: Pressure Negative					
R.R.	8.9	1.7			
H.S.	10.1	1.9			
H.S. (repeat)	9.5	9.8	50	10.5	8.8
H.P.	11.4	9.8			
L.L.	12.5	8.5			
Average	10.5	6.3			

RESULTS

The results of gas analysis of 19 cavities are presented in table 1. The cavities have been divided into three main categories: (*1*) open cavity with relatively free air or fluid flow through the communicating bronchi; (*2*) partially blocked cavity which had the characteristics of both open and blocked cavities in varying degrees. There was a transitory, incomplete or complete, hindrance of air flow through the communicating bronchi; (*3*) blocked cavity with no demonstrable free air or fluid flow through the communicating bronchi even though no difficulty was experienced in obtaining gas specimens.

In the wide open cavities, the mean initial pressure was between plus 2 and minus 2 cm. of water. The average carbon dioxide content was

3.5 volumes per cent and the oxygen content 17.8 volumes per cent. It is remarkable that the carbon dioxide content was never higher than in normal alveolar air (5.6 volumes per cent), nor was the oxygen content lower than in alveolar air (14 volumes per cent).

In the partially blocked cavity, the mean pressure varied between minus 4 and minus 18 cm. of water except in the one tension cavity with positive pressure. In 2 cases, the carbon dioxide content was markedly higher than in the first group. Oxygen content was similarly distinctly lower in 3 cases in this second group.

Blocked cavity results were striking: the carbon dioxide content was higher than the oxygen content.

Discussion

The results of gas analysis were, with a few exceptions, as anticipated. Although the air usually remains in the cavity for a longer time than in the alveoli, it does not show the same degree of alteration in a well-ventilated, open cavity as in the alveoli. This result is reasonable, because the main portion of the intracavitary air does not come in close contact with the cavity wall; the diffusion of gases through the cavity wall is slower than through the respiratory membrane; and in the cavity wall, the tuberculous process has often blocked the vessels of the pulmonary circulation which are then replaced by newly grown capillaries from the bronchial circulation. These, in turn, contain better oxygenated blood than the pulmonary arteries.

In the blocked cavity, air remains for a period of time which can be measured in hours or days. The level of carbon dioxide and oxygen, however, cannot be related to a static phenomenon since a simple equilibrium with the blood gases could not explain the high carbon dioxide and low oxygen values obtained. Ornstein, Herman, and Friedman (10) had the same experience when analyzing the air in artificial pneumothorax. It may be assumed that the tissues, cells, and microorganisms in the cavity wall partially cause this difference by their inherent aerobic metabolism. In exudative pneumothoraces, Grass and associates (11, 12) found an average carbon dioxide content of 8.64 volumes per cent with a peak of 13.62 volumes per cent; and an average oxygen value of 1.56 volumes per cent, i.e., comparable with the content of tuberculous cavities.

In the blocked cavity (Case *R.R.*), the above findings were confirmed. In this instance, a pulmonary cavity on the left and an old empyema cavity on the right were studied. The latter was apparently closed at the time of needling, and contained pus and air. The pulmonary cavity contained 8.91 volumes per cent of carbon dioxide and 1.70 volumes per cent of oxygen; and the empyema cavity, 10.45 volumes per cent of carbon dioxide and 1.69 volumes per cent of oxygen. These gas-containing spaces of different origin showed a striking similarity, especially in their oxygen content.

The blocked cavity of *H.S.* was needled twice. Six weeks after the first needling, some of the injected propyliodine was present. On the other hand, the contrast medium began to disappear in a few hours after the second needling. Bronchial blockade, which is almost always only temporary, was more constant in this case at the time of the first needling. As expected, gas analysis showed a much lower oxygen content on the first needling.

One somewhat contradictory result requires explanation. After aspiration of 300 cc. of air from a partially blocked cavity in patient *A.L.*, the carbon dioxide content increased from 3.7 volumes per cent to 8.9 volumes per cent. This patient had a large communicating cavitary system, probably with recesses which may not have been well aerated in spite of partially opened bronchi. It seems reasonable to believe that air currents followed the removal of air, and thus that the air samples probably came from different parts of the cavitary system.

The partially blocked cavities with negative pressure showed widespread variations in their carbon dioxide and oxygen contents. They resembled both open and blocked cavities, depending, of course, upon the duration and completeness of the bronchial blockade.

The gas obtained from the tension cavity was similar in content to the gas from cavities of the second group. This is reasonable because the tension cavity is a sort of partially blocked cavity since the bronchial communication opens only during inspiration.

The studies of the present writers confirm the classic works of Coryllos and his collaborators (2–4). They believed that the communicating bronchi were actually open although they might temporarily appear to be closed. In the present series, varying degrees of bronchial blockade have been seen. The communicating bronchi of the

blocked cavity which do not allow immediate air or fluid flow, however, may be patent some hours later. If it is clearly understood that the condition of the communicating bronchi can be only temporary, the classification of cavities into three groups as described is useful.

Coryllos also emphasized the effect of anoxemia in the closed or intermittently blocked cavity in slowing the rate of multiplication of tubercle bacilli within the cavity. It has become generally accepted that this is one of the principal mechanisms by which measures aimed at closure of tuberculous cavities achieve therapeutic results.

However, it has been suggested (13) that, if this concept is accepted, then blocking or intermittent closure of an active tuberculous cavity may be viewed as an obstacle to antimicrobial therapy. Sterilizing drugs, such as isoniazid and streptomycin, with effects that are exerted only against actively metabolizing tubercle bacilli under aerobic conditions (14), might be expected to have a slower antimicrobial action in blocked or partially obstructed cavities than in cavities with free bronchial communication and, therefore, high concentrations of oxygen.

Thus, it would appear that the availability of oxygen to tubercle bacilli *in vivo* may be as important a subject for contemporary investigation as it was in the pre-chemotherapy era.

Summary

Gas samples from 19 tuberculous cavities were obtained by direct transparietal needling and examined for their carbon dioxide and oxygen content.

Cavities were classified into three groups: (*1*) open cavity, in which air and fluid flowed freely through the communicating bronchi. The intracavitary pressure fluctuated around zero, and the gas composition varied between ordinary air and alveolar air; (*2*) partially blocked cavity with the gas content varying between the values of open and blocked cavities. The blockade of the communicating bronchi was either incomplete or ephemeral. (The initial pressure was either clearly negative or clearly positive (tension cavity)); (*3*) blocked cavity with initial negative pressure with a low oxygen content and a high carbon dioxide content. There was a total blockade of the communicating bronchi at the time of the needling, and this could not be broken by the withdrawal of the air from the cavity or by injection of propyliodine (Dionosil®) into the cavity.

It is believed that this classification should be related to the situation observed at the time of the examination because, in reality, almost all gas-filled cavities are anatomically open. Evidence was adduced that in a large and/or loculated cavity the composition of gas might be different in different parts.

The relevance of these studies to the antimicrobial therapy of tuberculosis is briefly discussed.

Sumario

Estudios del Contenido Gaseoso de las Cavernas Tuberculosas

Por medio de la punción transparietal directa con aguja obtuviéronse muestras de gas de diecinueve cavernas tuberculosas, examinándolas después en cuanto al contenido de bióxido de carbono y de oxígeno.

Las cavernas fueron clasificadas en tres grupos: Cavernas abiertas, en las que aire y líquido fluían con libertad a través de los bronquios de drenaje. La presión intracavitaria fluctuaba alrededor de cero y la composición del gas variaba entre aire ordinario y aire alveolar.

Cavernas parcialmente ocluídas cuyo contenido gaseoso variaba entre los valores de las cavernas abiertas y las ocluídas. El bloqueo de los bronquios de drenaje era ya incompleto o efímero. La presión inicial era ya netamente negativa o netamente positiva (caverna por tensión o c. insuflada).

Cavernas ocluídas de presión negativa inicial con contenido bajo de oxígeno y alto de bióxido de carbono. Había un bloqueo total de los bronquios de drenaje al hacer la punción, que no pudo deshacerse con la extracción del aire de la caverna o con la inyección de propilyodo (Dionosil®) en la caverna.

Opínase que esta clasificación debe relacionarse con la situación observada al hacer el examen, porque, en realidad, casi todas las cavernas llenas de gas están abiertas anatómicamente. Se aportan datos de que, en una caverna grande y/o loculada, la composición del gas podría ser distinta en diversas partes.

Se discute brevemente la relación que guardan estos estudios con la quimioterapia antimicrobiana de la tuberculosis.

Resume

Etude sur le contenu gazeux des cavernes tuberculeuses

Des échantillons d'air provenant de 19 cavernes tuberculeuses ont été obtenus par ponction transpariétale directe et leur teneur en CO_2 et en oxygène a été recherchée.

Les cavernes ont été classifiées en trois groupes:

Caverne ouverte, dans laquelle l'air et les liquides s'écoulaient librement par les bronches de drainage. La pression intracavitaire fluctuait autour de zéro et la composition des gaz variait entre l'air ambiant et l'air alvéolaire.

Les cavernes partiellement obstruées où la valeur du contenu gazeux varie entre celle des cavernes ouvertes et celle des cavernes obstruées. L'obstruction des bronches de drainage était incomplète ou passagère. La pression initiale était ou nettement négative ou nettement positive (cavité en tension).

La caverne obstruée qui a une tension négative initiale et une teneur réduite en oxygène et élevée en CO_2. Au moment de la ponction les bronches de drainage étaient complètement obstruées; l'extraction de l'air de la caverne ou l'injection de propyliodine (Dionosil)® dans la caverne n'a pas éliminé l'obstruction.

Les auteurs estiment que cette classification doit être mise en corrélation avec les conditions observées au moment de l'examen car, en fait, presque toutes les excavations remplies d'un mélange gazeux sont anatomiquement ouvertes. On a apporté la preuve que dans une excavation large ou cloisonnée la composition gazeuse pouvait être différente en des points différents.

Le rapport entre ces études et la chimiothérapie antituberculeuse est brièvement commenté.

Addendum

Since acceptance of this paper for publication, a fatality has occurred. A patient, studied in December, 1958, had a pneumothorax as a result of the procedure described in this report and died one week later. The details of this experience will be described in a separate communication. Suffice it to point out that needling of pulmonary cavities is not without hazard. Further application of the procedure described here is contemplated only when preliminary investigation has established that pleural symphysis exists.

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ANALYSIS AND LATE RESULTS OF RESECTION FOR PULMONARY TUBERCULOSIS IN HAWAII[1]

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(Received for publication February 2, 1959)

INTRODUCTION

The present report consists of a detailed analysis of all of the resections for pulmonary tuberculosis at Leahi Hospital, Honolulu, between August, 1946, and January, 1957, with follow-up observations ranging from two to more than twelve years.

Composition of Series

Eight of 357 patients had bilateral resection or more than one operation. Resection was not done in 3 patients because it was considered inadvisable after opening the chest. These patients are now well and are not included in the study, which therefore numbers 354. The follow-up period averages more than six years, 75 per cent of the group having been followed for more than four years and only 4 per cent having been followed for the minimal period of two years. The range of ages was seventy-two to two years; one-third of the patients were between twenty and thirty years of age; one-third were more than forty, and only 23 (14 per cent) were twenty years of age or younger.

OBSERVATIONS

In 59 per cent of the patients, reversal of infectiousness was accomplished by the operation. In 6 per cent, tubercle bacilli persisted in the sputum after operation, and the tuberculous lesion on the operated side seemed to be the source. If a patient had sputum negative for tubercle bacilli for eight or more months before operation, he was considered "negative," which was true in 28 per cent of the patients. Of the whole group, 84 per cent originally had tubercle bacilli demonstrated in the sputum by microscopy or culture at the onset of hospitalization.

In other ways the present study differs from others that have been reported: The entire follow-up study has been made by the writers and is complete. Most of the patients have been in constant island residence. Only 6 (1.6 per cent) were lost after follow-up study of approximately two years; only 15 (4.2 per cent) were classed as unsanctioned discharges. Very few of the patients were military veterans, and 42 per cent were females. Virtually all of the operations were supervised or performed by the senior author, and pre- and postoperative care was under his daily supervision. He agreed with the decision to advise surgical treatment in every case.

The ethnic distribution was approximately as follows: Japanese, 55 per cent; Filipino, 11 per cent; Hawaiian and part Hawaiian, 9 per cent; Chinese, 6 per cent; United States or European, 5 per cent; mixed and other national origins, 14 per cent.

The first 8 operations were performed without accompanying chemotherapy. Four were followed by tuberculous complications. These 8 patients are all well twelve years after the surgery. The period of study includes the early days of drug usage in the treatment of tuberculosis and permits a comparison of a variety of pre- and postoperative regimens.

The pleasant, lightly variable temperature, sunny unpolluted air, and the measure of disregard for self and family stress in the usual island way of living make most patients cooperative and satisfied with their lot. They rarely leave the hospital against advice or refuse surgical treatment and shoulder their hardships sensibly and cheerfully.

In table 1 the type of surgical procedure is divided into eight categories and the distribution of deaths, complications, and late results are recorded. Many patients had multiple complications, but in the table only one—the most significant—complication is listed. In the whole group the number of tuberculous complications is undoubtedly influenced by the fact that 166 patients had preoperative chemotherapy for six weeks or less.

In general the extent of the operative procedure paralleled the extent of the disease; both contributed heavily to the risks of the operation and of the disease. Thus, there is a definite tendency for the untoward happenings to increase with the extent of the surgical procedure,

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TABLE 1

TYPE OF SURGICAL PROCEDURE AND DISTRIBUTION OF DEATHS, COMPLICATIONS, AND LATE RESULTS

Procedure	Number of Patients						Nontuberculous Operative Complications	All Tuberculous Complications. Major and Minor. Either Side.		Late Cor Pulmonale	Late Tuberculous Deaths	Late Nontuberculous Deaths
	Operated	Readmitted	Now Hospitalized	On Chemotherapy	Well. No Treatment. Able to Work	Operative Deaths		Early	Late			
Pleuropneumonectomy and pneumonectomy	59	9	0	4	49	3	1	8	8	1	0	3
Bilobectomy and lobectomy plus	26	2	0	1	19	3	1	4	1	1	2	1
Lobectomy	86	13	1	2	78	2	7	4	16	1	0	3
Two segments plus	9	2	0	0	8	0	0	0	2	0	0	1
Two segments	49	3	2	0	45	0	7	2	4	0	0	2
Single segment plus	24	4	0	2	22	0	1	2	3	0	0	0
Single segment	69	6	1	1	66	0	7	3	8	1	0	1
Subsegment, local excision, wedge	32	0	0	0	30	0	3	0	1	0	0	2
Totals	354	39	4	10	317	8	27	23	43	4	2	13
Per cent					89	2.2	7.6	6.5	12	1	0.5	

Risk of Operation

Risk of Disease and Disease Plus Operation

30%

although the small numbers in each category make statistical significance questionable.

Despite the somewhat more difficult postoperative management and the higher earlier complication rate of partial lobectomy, and in the face of a growing popularity for lobectomy, it is believed that preservation of uninvolved lung tissue should be uppermost in the surgeon's mind. It is likewise believed that the nature and the extent of the disease should determine the type of operative procedure. This is especially the case because the present analysis indicates that further late disability from tuberculosis is at least equally as common after lobectomy as it is after partial lobectomy.

The desire to conserve lung tissue was particularly influenced by the state and degree of therapeutic control and the status of the residual and the other lung. Thus, preservation of an anterior upper lobe segment containing small firm nodules in an elderly patient with contralateral scar and some emphysema is obviously preferable to lobectomy if the chemotherapeutic control could be considered to be reasonably satisfactory. If not, lobectomy would be indicated unless the patient's pulmonary function was markedly borderline. Certainly, lobectomy should not be chosen when a lesser resection is feasible, because all other lobes are uninvolved and the patient is an excellent risk, unless there is a strong chemotherapeutic reason.

It is of interest that, in the late deaths, cor pulmonale outnumbered tuberculosis.

The first 8 resections were performed before streptomycin was available. As mentioned before, 4 (50 per cent) of these patients had tuberculous complications, but eventually made complete recoveries. There followed a series of 15 pneumonectomies and one lobectomy performed with associated therapy with streptomycin alone, given for a period of four days to two weeks in a total dosage of only about 8 to 12 gm. In this group one late contralateral reactivation and one empyema (12 per cent) were present. The value of the drug was immediately apparent, but the implication that such short administration was adequate was contradicted by further experience. With medical as well as with surgical

TABLE 2

COMPLICATIONS

Bronchopleural fistula	7
Tuberculous empyema	6
Nontuberculous empyema	1
Tuberculous wound infection	7
Early tuberculous reactivation or spread on the same side	7
Thoracoplasty for early reactivation	6
Early tuberculous reactivation or spread on the other side	4
Recurrent tuberculous tracheobronchitis	5
Postoperative atelectasis	5
Pleural pocket requiring reinstituted drainage or reoperation	7
Early thoracoplasty for residual pleural pocket	6
Hemorrhage requiring reoperation	3
Hemorrhage requiring secondary transfusion	3
Reoperation for bronchostenosis	2
Reoperation for lost needle	1
Reoperation for temporary arterial ligature	1
Severe blood transfusion reaction	2

TABLE 3

CAUSES OF DEATH

Operative Deaths:	
Hemorrhage	2
Respiratory—acidosis, failure, and asphyxia	4
Drug reaction	1
Transfusion reaction	1
Late deaths:	
Tuberculosis	2
Cor pulmonale	3
Injury	2
Leukemia	2
Blood transfusion	1
Uremia	1
Cerebral hemorrhage	1
Pneumonia	1
Pulmonary embolism	1
Retroperitoneal hemorrhage	1

patients, there was a gradual increase in the duration of chemotherapy to six weeks, to four months, to eight months, to a year, and even longer.

This variety of 73 complications includes some of the operative deaths and was experienced by 61 patients. Four of these had no preoperative chemotherapy and provided 8 of the complications. Thoracentesis for small residual air or fluid pockets following drainage tube removal is considered a noncomplicating postoperative incident. This procedure was performed in 6 patients.

A major object of this analysis was to seek the causes of further disabilities from tuberculosis—early or late—following pulmonary resection. For this purpose, the operative deaths were excluded because they were not related to the disease and there was no opportunity for follow-up observation. Patients who had little or no preoperative chemotherapy were also excluded. Only the "highlights" of the analysis of the remainder of the group are presented.

Preoperative Tuberculosis Chemotherapy

Presurgical treatment	Percentage of patients with tuberculous complications
4 to 6 weeks	28
↓	↓
8 to 12 months	0
Third course or more	30
↓	↓
First course	10
Streptomycin only	36
Streptomycin plus	10

Only 8 per cent of these patients who received four to eight months of chemotherapy preceding surgery experienced further difficulties with tuberculosis. Treatment for four months or less, however, was associated with a complication rate of 18 per cent. Sixty-three per cent of the group with complications and only 30 per cent of the patients with an uncomplicated course had four to six weeks of presurgical treatment. At the time of surgery about half the group without complications and less than a fourth of the patients with complications were in their first course of chemotherapy. Only 28 per cent of those without complications and 65 per cent of those with complications received streptomycin alone as their sole preoperative chemotherapy. It is believed that the ideal preoperative chemotherapy consists of the administration of more than one drug for approximately eight months.

Postoperative Tuberculosis Chemotherapy

Postsurgical Treatment	Percentage of patients with tuberculous complications
6 weeks or less	35
↓	↓
8 to 12 months	4
Streptomycin only	35
Streptomycin plus	16

Forty per cent of the patients without complications and 78 per cent of those with complications had postoperative chemotherapy for four months or less. By contrast, 60 per cent of those without complications and only 22 per cent

of those with complications had chemotherapy for so long as four months to more than a year. Considering only the late tuberculous reactivations or spreads on either side, 83 per cent of the entire group had postsurgical chemotherapy for four months or less, and only 9 per cent received drug treatment for eight months to more than a year. It is believed that the ideal postoperative chemotherapy should consist of multiple-drug regimens administered for at least eight months to more than a year.

Bacteriologic Studies

Feature	Percentage of patients with tuberculous complications
Specimen culture positive for tubercle bacilli	22
Specimen culture negative for tubercle bacilli	4
Preoperative tubercle bacilli drug resistant or partially drug resistant	32
Preoperative tubercle bacilli drug susceptible	16
Sputum "conversion" by operation	16
Sputum negative for tubercle bacilli for eight months before and continued negative after operation	3

Bacteriologic studies of the surgical specimens were done in 60 per cent of this series. In the patients without complications the resected specimens were negative on culture for tubercle bacilli in 73 per cent and positive in 27 per cent. These figures were reversed to 33 per cent and 67 per cent, respectively, in the group with postoperative complications.

The combination, considered unfavorable, of a lung specimen from which tubercle bacilli could be cultured and postoperative chemotherapy for six weeks or less was rare (2.6 per cent) in the uncomplicated group, but common (21 per cent) in the complicated group. Some loss of the drug susceptibility of the patients' tubercle bacilli occurred in 16 per cent of the patients with an uncomplicated course and in 36 per cent of those with postoperative complications.

Of those patients with tubercle bacilli in the sputum at the time of operation, reversal of infectiousness occurred in 60 per cent of the group without complications and in 49 per cent of the group with complications. Sixteen per cent of these patients with "operative conversions" had later difficulties with tuberculosis. On the other hand, sputum "conversion" was effected by preoperative chemotherapy in 37 per cent of the "uncomplicated" group and in only 6 per cent of the patients with postoperative complications.

It is concluded that a positive specimen culture is an admonition to prolong and possibly augment postoperative chemotherapy. At least partial drug susceptibility of the patients' strain of tubercle bacilli is highly desirable for all patients and is mandatory in the "advanced risk" cases. While failure of the sputum to become free of tubercle bacilli following operation is an unfavorable sign, the *disappearance* of the bacilli in such circumstances is not nearly as favorable as when the disappearance has occurred some months before operation.

Morbid Anatomy and Complicating Conditions

There were few differences between the two groups with and without complications when the pathologic changes of the resected specimens were classified. The percentage of complications was about the same in each group for a single open cavity, multiple cavities, filled cavity, and caseous nodules with and without liquefaction. More specimens from the group with postoperative complications were classed as destroyed; more from the "uncomplicated" group were classified as predominantly clustered nodules. Gross bronchial involvement was twice as common in the patients with postoperative complications. In 11 patients, healing appeared to be complete or almost complete. In only 6 were cystlike structures excised that were thought to represent completely healed cavities.

The pressure of diabetes did not seem to affect the possibility of attaining an uncomplicated lasting result. Involvement of the larynx and larger bronchi was more than twice as frequent in the group with postoperative complications. Less than 1 per cent of the "uncomplicated" group and 21 per cent of the "complicated" group had contralateral tuberculous disease recently considered to be cavitary. The occurrence of contralateral disease considered to be under control was the same for both groups. By contrast, too few patients had notable emphysema to make a good comparison, although it was three times as frequent in the patients with complications.

Eighty-one per cent of the patients with "recently open" or "open" contralateral disease had further tuberculous disabilities.

Hospitalization

Forty-three per cent of the "uncomplicated" group and 51 per cent of the patients with complications had been previously hospitalized for tuberculosis—a smaller difference than might have been expected in view of the general feeling that "repeaters deserve a less favorable prognosis."

In this series, except for the presence of fever, bed rest has seldom been emphasized. Early ambulation and pre- and postoperative exercises have always been the rule. There has been an unregretted, constantly progressive tendency to hasten the return of full physical activity and recovery.

In the "uncomplicated" group, 24 per cent of the patients were discharged within six months after operation and 87 per cent within a year. In the group with complications these figures were 9 per cent and 28 per cent. This is not really a fair comparison because hospitalization was naturally prolonged in the group with complications. However, early discharge cannot be blamed for readmission after surgery, for only 18 per cent of the readmitted patients had had less than six months of postoperative hospitalization. Of those readmitted primarily for late tuberculous reactivation, 22 per cent, almost the same as the "uncomplicated" group (24 per cent), had had only six months of postoperative hospitalization or less.

The remaining lung on the operated side was the site of a tuberculous reactivation that necessitated readmission twice as frequently as occurred on the contralateral side. Such readmissions occurred in the first three years in 70 per cent of the entire group. In 20 per cent, the readmission took place five years after the operation. A gradual decline in the readmission rate was noted from year to year, three occurring after six years and one after seven years.

While this study clearly indicates the value of lengthy multiple-drug postoperative chemotherapy, it is believed that in favorable cases postoperative hospitalization need not exceed four to six months. Certainly, it should never be prolonged in an attempt to include the entire period during which reactivation of the tuberculosis might occur.

Summary

An analysis has been made of the results obtained from excisional surgery of pulmonary tuberculosis in a group of 357 patients treated at Leahi Hospital, Honolulu. The evaluation supports the general realization that the nature and volume of the untoward results parallel the extent and state of the disease. The results further indicate that, even with only partial drug susceptibility of the patients' tubercle bacilli, resectional surgery may afford salvage of useful life and mitigation of illness only less miraculous than that of chemotherapy itself. In the proper management of pulmonary tuberculosis, a regimen of chemotherapy should be initiated with the hope of rapidly bringing the patient to a point where surgical excision is unnecessary or offers the best opportunity for complete recovery and shortened hospitalization.

Inadequate pre- and postoperative chemotherapy and less than a meticulous selection of patients for surgical treatment produced a 30 per cent rate of untoward incidents related to the operation or the disease. On the other hand, surgical excision was completely successful if the following features were present: if multiple-drug preoperative chemotherapy was the initial chemotherapy for tuberculosis and exceeded six to eight months; if the contralateral lung was normal; if the response to chemotherapy was rapid and favorable, with sputum conversion; if the original cultures displayed drug susceptibility and culture of the resected specimen yielded no tubercle bacilli; and if the multiple-drug therapy was continued postoperatively—in or out of the hospital—for at least a year.

Sumario

Análisis y Resultados Tardíos de la Resección por Tuberculosis Pulmonar en Hawai

Comprende este análisis los resultados obtenidos con la cirugía excisional de la tuberculosis pulmonar en un grupo de 357 enfermos tratados en el Hospital Leahy de Honolulu. Esta apreciación apoya la comprensión general de que la naturaleza y el volumen de los resultados adversos reflejan la extensión y el estado de la enfermedad. Indican además los resultados que, aun no habiendo más que farmacosusceptibilidad parcial de parte de los bacilos tuberculosos del enfermo, la resección quirúrgica puede proporcionar una salvación de vida útil y mitigación de enfermedad, apenas menos milagrosas que las de la quimioterapia misma. En la asistencia adecuada de la

tuberculosis pulmonar, debe iniciarse un régimen quimioterapéutico con la esperanza de llevar rápidamente al enfermo a un punto en que sea innecesaria la excisión quirúrgica o que ofrezca la mejor oportunidad para la curación absoluta y el acortamiento de la hospitalización.

La inadecuada quimioterapia pre- y postoperatoria y una selección que no cabe llamar meticulosa de los enfermos para tratamiento quirúrgico produjo un coeficiente de 30 por ciento de incidentes adversos relacionados con la operación o la enfermedad. En cambio, la excisión quirúrgica obtuvo éxito absoluto cuando se cumplían las siguientes condiciones: la poliquimioterapia preoperatoria constituía la quimioterapia inicial para la tuberculosis y había durado más de seis a ocho meses; el pulmón contralateral era normal; la respuesta a la quimioterapia era rápida y favorable con viraje del esputo; los cultivos primitivos desplegaban farmacosensibilidad y el cultivo del ejemplar resecado no rendía bacilos tuberculosos; y se continuaba la politerapia postoperatoriamente—dentro y fuera del hospital—a lo menos un año.

Resume

Analyse et résultats tardifs de l'exérèse pour tuberculose pulmonaire en Hawai

On a effectué une analyse des résultats obtenus par chirurgie d'exérèse dans la tuberculose pulmonaire chez un groupe de 357 malades traités à l'hôpital Leahi, à Honolulu. L'évaluation vient à l'appui de l'opinion générale qui estime que la nature et l'importance des résultats défavorables sont comparables à l'étendue et au stade de la maladie. Les résultats indiquent en outre que même lorsque la sensibilité aux drogues des bacilles tuberculeux du malade n'est que partielle, la chirurgie d'exérèse, qui permet de sauver une vie utile ou d'atténuer la maladie, n'est pas moins miraculeuse que la chimiothérapie elle-même. Dans un traitement approprié de la tuberculose pulmonaire un programme de chimiothérapie doit être instauré avec l'espoir d'amener rapidement le malade à un état où l'excision chirurgicale n'est pas nécessaire ou offre la meilleure chance d'obtenir une guérison complète avec une période d'hospitalisation plus courte.

Une chimiothérapie inadéquate pré- et postopératoire et une sélection insuffisamment rigoureuse des malades destinés à être opérés ont procuré un taux d'incidents défavorables de 30% en rapport avec l'intervention ou avec la maladie. D'autre part, l'excision chirurgicale a procuré un succès complet quand les conditions suivantes ont été remplies: lorsque la chimiothérapie préopératoire à l'aide de drogues multiples représentait la chimiothérapie initiale pour tuberculose et avait duré plus de six à huit mois; lorsque le poumon opposé était normal; lorsque la réponse à la chimiothérapie était rapide et favorable avec négativation des crachats; lorsque les cultures originales montraient une sensibilité à la drogue et que la culture des fragments réséqués ne contenait pas de bacilles tuberculeux; lorsque le traitement à drogues multiples était poursuivi après la résection soit à l'hôpital, soit au dehors, pendant une année.

A FACTOR IN SERUM WHICH PREVENTS PROPAGATION OF D-29 MYCOBACTERIOPHAGE WITH HUMAN TUBERCLE BACILLI[1, 2, 3]

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(Received for publication February 20, 1959)

Introduction

Fresh human serum in concentrations of 10 per cent and greater has been found to prevent propagation of D-29 mycobacteriophage with the human virulent strain, *M. tuberculosis* H37Rv, and its avirulent variant H37Ra, but did not prevent propagation of phage with the saprophyte *Mycobacterium sp.* ATCC 607. The factor is relatively stable and has been found to be concentrated in a fraction of serum which precipitates when 33 per cent by volume of a saturated solution of ammonium sulfate is added (the gamma globulin fraction).

The factor is distinct from another fraction in serum previously described in this laboratory (1) which allows D-29 phage to propagate in the presence of concentrations of sorbitan monooleate (Tween® 80) which would otherwise prevent adsorption of phage and subsequent propagation. The latter factor has been found to be concentrated in the albumin fraction of serum and will be the subject of a separate communication (2). The present report will describe the antiphage propagation factor in serum and some of its properties.

Materials and Methods

Propagation of D-29 mycobacteriophage: The suspensions of D-29 mycobacteriophage[5] used in these experiments were prepared by adding 10 ml. of a ninety-six-hour broth culture of *Mycobacterium sp.* ATCC 607 and 2 ml. of a stock phage suspension to 300 ml. of heart infusion broth.[6]

After incubation at 37° C. for forty-eight hours, the broth was filtered through a Seitz filter and stored at 4° C. as stock phage suspension. Titers of phage in these suspensions usually reached 10^9 plaques per ml. Refiltration of suspensions may be needed to remove strain ATCC 607. Contamination with this culture will void experiments with tubercle bacilli.

Enumeration of plaques of D-29 mycobacteriophage: One-tenth of a milliliter of appropriate dilutions of substances to be tested were incorporated into 3 ml. of semi-solid 0.8 per cent heart infusion agar overlayer with 0.5 ml. of a ninety-six-hour broth culture of *Mycobacterium sp.* ATCC 607. After incubation for forty-eight hours at 37° C., counts were made of the plates containing the largest number of discrete plaques and this number was multiplied by the appropriate dilution factor to obtain the plaque count.

Effect of serum and serum fractions on phage propagation: These tests were performed by adding the constituents listed below to test tubes 180 by 22 mm., mixing, and withdrawing aliquots for plaque counts before and after incubation for forty-eight hours at 37° C.

One milliliter of a ninety-six-hour culture of *Mycobacterium sp.* ATCC 607 homogenized in a Teflon grinder in heart infusion broth, or a fourteen-day-old homogenized culture of H37Rv or H37Ra[7] in Dubos broth base[8] with albumin but without sorbitan mono-oleate or oleic acid.

Four milliliters of fresh serum or serum preparation diluted in the broth specified above to provide the appropriate final concentration. Fresh serum or serum fractions, unless otherwise identified, were obtained from donors *W*, *K*, *F*, and *M* (table 1) and used without distinction.

Sufficient calcium chloride, U.S.P. ($CaCl_2 \cdot 2H_2O$) to provide an added concentration of 0.001 moles per liter of Ca^{++}. (This was added because tests in this laboratory revealed that this concentration of added calcium allowed the greatest yields of D-29 phage with tubercle

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[1] From the George Hunter Laboratory, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee.

[2] This study was supported by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association, and from the Tennessee County Tuberculosis Associations.

[3] Presented in part before the Medical Session, as part of *Section 3B*, at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 26, 1959.

[4] Present address: Department of Medicine, University of Louisville Medical School, Louisville, Kentucky.

[5] D-29 mycobacteriophage and its propagating strain, ATCC 607, were obtained from Dr. Seymour Froman, Olive View Sanatorium, Olive View, California.

[6] Heart infusion broth was obtained from Difco Laboratories, Detroit, Michigan.

[7] *M. tuberculosis* H37Rv and H37Ra were obtained from Dr. William Steenken, Trudeau Laboratories, Saranac Lake, New York.

[8] Dubos broth base and albumin were obtained from Difco Laboratories, Detroit, Michigan.

TABLE 1

PROPAGATION OF D-29 PHAGE WITH M. TUBERCULOSIS H37Rv IN HUMAN SERUM FROM SEVERAL SOURCES, BEFORE AND AFTER TREATMENT WITH TRYPSIN

Serum Source	Treatment with Trypsin	Serum Concentration (Per Cent)									
		52.0	26.0	13.0	10.0	6.5	5.0	3.2	2.5	1.6	1.2
Tuberculin negative	F No	10^{1}*	10^{1}	0		10^{7}		10^{7}		10^{8}	
	Yes	10^{6}	10^{6}	10^{7}		10^{8}		10^{8}		10^{8}	
	M No	—	10^{1}	10^{2}		10^{2}		10^{5}		10^{7}	
	Yes	—	10^{6}	10^{6}		10^{6}		10^{6}		10^{8}	
Tuberculin positive	K No	10^{1}	10^{2}	10^{1}		10^{6}		10^{7}		10^{8}	
	Yes	10^{7}	10^{8}	10^{8}		10^{8}		10^{8}		10^{8}	
	W No	0	0	10^{1}		10^{2}		10^{5}		10^{8}	
	Yes	10^{6}	10^{6}	10^{7}		10^{8}		10^{8}		10^{8}	
Acute pulmonary tuberculosis	1 No				10^{2}		10^{2}		10^{6}		10^{9}
	Yes	10^{7}	10^{8}	10^{8}		10^{8}		10^{7}		10^{7}	
	2 No				10^{2}		10^{6}		10^{8}		10^{8}
	Yes	10^{8}	10^{6}	10^{9}		10^{8}		10^{8}		10^{8}	
	3 No				10^{2}		10^{5}		10^{9}		10^{9}
	Yes	10^{9}	10^{8}	10^{8}		10^{8}		10^{8}		10^{7}	
	4 No				10^{2}		10^{7}		10^{9}		10^{9}
	Yes	—	10^{8}	10^{9}		10^{9}		10^{9}		10^{8}	
	5 No				10^{2}		10^{5}		10^{9}		10^{9}
	Yes	10^{6}	10^{8}	10^{9}		10^{8}		10^{9}		10^{8}	

* Plaques per ml.

Initial phage concentration: 100 to 800 plaques per ml.

Propagating strains: *M. tuberculosis*, H37Rv, 1 ml., undiluted, homogenized culture

Controls:

Propagating system and trypsin yielded 1×10^{8}

Propagating system without serum yielded 1×10^{8} plaques per ml.

bacilli in the absence of serum when compared with results in media with several other concentrations of added calcium or without it.)

One-tenth of a milliliter of 10^{-4} dilution of stock suspension of D-29 phage.

Effect of trypsin on the serum factor: Fresh human serum was incubated with 1,066 units per ml. of crystalline bovine trypsin[9] at 37° C. for one hour, followed by heating at 56° C. for one-half hour to inactivate the enzyme. This material was then tested for its effect on propagation of phage. In control tests it was found that the addition of 5,000 units per ml. of trypsin to propagation systems without serum did not interfere with phage propagation.

Effect of M. tuberculosis H37Rv, Mycobacterium sp. ATCC 607, or D-29 phage on the serum factor: To 8 ml. of fresh serum were added 2 ml. of cultures of *Mycobacterium sp.* ATCC 607, *M. tuberculosis* H37Rv, or a stock suspension of D-29 phage. The mixtures were incubated for one or two days at 37° C. After filtration through a Seitz filter, the antiphage propagation potency of these preparations was compared with that of serum incubated with broth alone.

[9] Bovine trypsin (Tryptar®), Armour and Company, Chicago, Illinois.

Effect of serum on the survival of D-29 mycobacteriophage: Suspensions of undiluted D-29 phage were incubated at 37° C. in Dubos broth with added calcium with, and without, 80 per cent serum. Plaque counts were made of aliquots withdrawn at one-half, one, two, four, and eighteen hours.

Effect of serum on the removal of phage from suspension by centrifugation of a propagating system: One milliliter of a homogenized culture of *M. tuberculosis* H37Rv was added to 3 ml. of Dubos broth with 0.001 moles per liter of added calcium and 1 ml. of serum, and to other tubes containing only 4 ml. of broth with added calcium. The tubes were incubated at 37° C. for two hours, after which 0.1 ml. of 10^{-4} dilution of phage was added to the tubes. These were further incubated for one-half hour, and plaque counts were made of aliquots of the liquid phase of each tube before and after centrifugation at 3,000 r.p.m. for ten minutes.

The tubes were then incubated for a further period of forty-eight hours, and plaque counts were made to confirm the antiphage propagation potency of the serum.

Preparation of serum fractions by precipitation with ammonium sulfate: To approximately 100 ml. of fresh serum was added a volume of saturated

solution of ammonium sulfate equal to one-third of the final volume. After sitting one-half hour at 4° C., a precipitate which formed was removed by centrifugation and filtration. By a similar procedure, precipitates were obtained by the addition of a saturated solution of ammonium sulfate to the first supernatant to constitute 55 per cent of the final volume, and subsequently by complete saturation of the second supernatant with ammonium sulfate crystals.

The three precipitates were individually dissolved in approximately 20 ml. of a phosphate buffer ionic strength 0.2, pH 7.8 in a cellophane bag, and dialyzed against frequent changes of the same buffer for forty-eight to seventy-two hours at 4° C.

The dialyzed fractions in phosphate buffer were filtered through a Seitz filter and assayed for antiphage propagation potency as described above. The three fractions were tested by paper electrophoresis and found to consist principally of the fractions designated.

Results

Effect of Serum on Propagation of D-29 Mycobacteriophage

Data are shown in table 1 which reveal that phage propagation as determined by plaque formation did not occur in tests containing serum from tuberculin-positive and tuberculin-negative donors, and from patients with acute pulmonary tuberculosis, in concentrations of 10 per cent and greater when the propagating strain was H37Rv. In table 2 it may also be seen that propagation did not occur when H37Ra was used as the propagating strain. In contrast, propagation proceeded to high titers through a wide range of serum concentrations when ATCC 607 was used as a test organism.

TABLE 2

Effect of Fresh Human Serum on the Propagation of D-29 Mycobacteriophage with M. Tuberculosis H37Rv, H37Ra, and Mycobacterium sp. ATCC 607

Per Cent Serum	Plaques per Ml.							
	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	H37 Rv	607	H37 Rv	607	H37 Rv	H37 Ra	H37 Rv	H37 Ra
80.0	0	10^8						
40.0	0	10^8	10^1	10^8	10^1	0	10^1	10^1
20.0	10^1	10^9	10^2	10^9	10^1	10^1	10^1	10^2
10.0	10^1	10^9	10^1	10^9	0	10^2	10^2	10^2
5.0	10^2	10^9	10^2	10^9	10^4	10^8	10^7	10^4
2.5	10^8	10^9	10^7	10^9	10^7	10^9	10^8	10^8
1.2	10^8	10^9	10^8	10^{10}	10^8	10^8	10^8	10^8

Initial phage concentration: 40 to 400 plaques per ml.

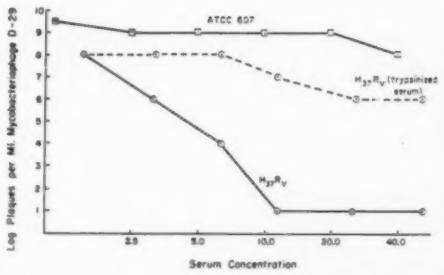

Fig. 1. Average values of the two experiments with *Mycobacterium sp.* ATCC 607 (*see* table 2) are shown with the median values of the experiments with the 4 tuberculin-negative and -positive donors (*see* table 1). D-29 phage propagated well in a wide range of serum concentrations with ATCC 607, whereas plaque counts in fresh serum concentrations of 10 per cent or more actually diminished slightly in media containing H37Rv. Trypsin inactivated this factor of serum.

These results are described graphically in figure 1 where median values from tests with the serum of tuberculin-negative and -positive donors are plotted before and after treatment with trypsin. The high titers obtained with propagation on ATCC 607 are also shown.

In figure 2 the inhibition of phage propagation is indicated by the growth of H37Rv colonies in the area of diffusion of serum (*A*) from a small steel cylinder placed on the surface of an agar plate. The H37Rv and D-29 phage were seeded in a semi-solid overlayer on the plate. In *B*, an aliquot of the same serum was added except that it was treated with trypsin previously, and no visible growth of H37Rv occurred, presumably as a result of the action of D-29 in the semi-solid overlayer.

Effect of Serum Fractions on Phage Propagation

In table 3, three experiments are shown in which serum fractions ranging in concentration from 1.25 to 40 per cent were added to D-29–H37Rv propagation systems. These fractions represented one-fourth to one-third (see table) the volume of the whole serum from which they were derived. It may be seen that, uniformly, tests containing 10 per cent or more of the gamma

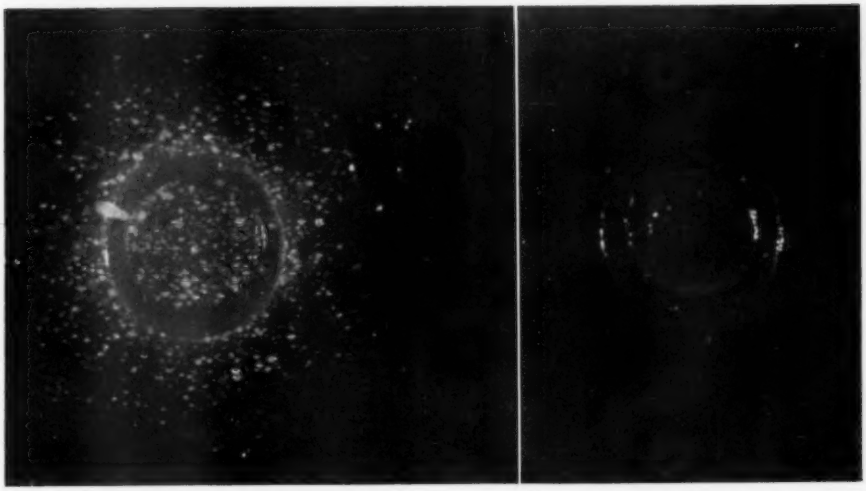

FIG. 2A (Left). The surface of a semi-solid agar overlayer inoculated with H37Rv and D-29 phage and incubated for twenty-eight days at 37° C. The circular indentation is the imprint of a small cylinder (penicylinder, inside diameter, 8 mm.) which was filled with fresh serum. Note the numerous colonies of H37Rv in the area of serum diffusion. (Magnified 4 ×)

FIG. 2B (Right). A preparation similar to Fig. 2A except that the cylinder was filled with serum previously treated with trypsin. D-29 phage has almost completely prevented growth of H37Rv. (Magnified 4 ×)

TABLE 3

EFFECT OF SERUM FRACTIONS ON PROPAGATION OF D-29 PHAGE WITH M. TUBERCULOSIS H37Rv

Serum Fraction Per Cent	33 Per Cent Saturation (Gamma Globulin)			33–55 Per Cent Saturation (Alpha and Beta Globulin)			55–100 Per Cent Saturation (Albumin)		
	Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3
	Ml. of Fraction Obtained per Ml. of Whole Serum			*Ml. of Fraction Obtained per Ml. of Whole Serum*			*Ml. of Fraction Obtained per Ml. of Whole Serum*		
	0.33	0.23	0.28	0.33	0.23	0.28	0.67	0.28	0.43
40.0	10^{2}*	10^{2}	10^{2}	10^{5}	10^{1}	10^{6}	10^{7}	10^{8}	10^{8}
20.0	10^{2}	10^{2}	10^{2}	10^{6}	10^{1}	10^{6}	10^{8}	10^{8}	10^{8}
10.0	10^{2}	10^{2}	10^{2}	10^{6}	10^{1}	10^{8}	10^{8}	10^{8}	10^{8}
5.0	10^{8}	10^{2}	10^{2}	10^{7}	10^{2}	10^{8}	10^{7}	10^{8}	10^{8}
2.5	10^{8}	10^{4}	10^{5}	10^{8}	10^{4}	10^{8}	10^{7}	10^{8}	10^{8}
1.3	10^{8}	10^{7}	10^{8}	10^{8}	10^{8}	10^{8}	10^{8}	10^{8}	10^{8}

* Plaques per ml. of D-29.

globulin fraction inhibited phage propagation. In one test with the alpha and beta globulin fraction in concentrations of 10 to 40 per cent, phage did not propagate. In two others, substantial propagation occurred in these concentrations of the fraction. In tests with albumin, no inhibition of propagation was observed.

Effect of Serum on the Removal of Phage by Centrifugation of a Phage–H37Rv Propagation System

In this study, twenty paired experiments were performed in which 20 per cent or no serum was present in propagation systems in Dubos broth. Plaques were enumerated before and after cen-

TABLE 4

EFFECT OF SERUM ON PHAGE ADSORPTION BY H37Rv

20 Per Cent Serum			No Serum		
Plaques per Ml.		Removal Per Cent	Plaques per Ml.		Removal Per Cent
Before Centrifugation	Supernatant		Before Centrifugation	Supernatant	
68	29	57.4	96	70	27.1
81	24	70.4	103	67	35.0
82	46	43.9	142	102	28.2
83	36	56.6	163	103	36.8
89	67	24.7	176	121	31.2
93	28	69.9	101	61	39.6
100	68	32.0	144	93	35.4
103	23	77.7	124	66	46.8
103	25	75.7	73	36	50.7
111	55	50.4	127	57	55.1
116	64	44.8	141	93	34.0
131	64	51.1	151	135	10.6
132	27	79.5	124	91	26.6
161	86	46.6	117	61	47.9
186	34	81.7	162	105	35.2
189	114	39.7	144	74	48.6
208	130	37.5	183	130	29.0
211	118	44.1	152	77	49.3
231	115	50.2	167	106	36.5
1,270	260	79.5	1,410	1,010	28.4
Average 187.4	70.6	62.3	200.0	132.9	33.6

Significance of difference between tests with serum and no serum: P = <0.001 (t-test) and 0.002 (Wilcoxon signed-rank test).

trifugation, and the results are described in table 4. There is reasonable agreement in the plaque counts which were theoretically identical in the tests before centrifugation. Following centrifugation, there was a very significant reduction in counts from tests in which 20 per cent serum was present. In contrast, in tests with no serum, only a moderate loss of phage from the supernatant occurred. The variation in these results is appreciable and is greater than that which occurred in similar tests with ATCC 607 (1). Nevertheless, the results are highly significantly different (P = <0.001, t-test, and 0.002 Wilcoxon signed-rank test).

Effect of Heat, Storage, and Incubation with M. Tuberculosis H37Rv, ATCC 607, or D-29 Mycobacteriophage on the Potency of the Antiphage Propagation Factor

It was found that storage of serum at −4° C., at 4° C. for four months, at 37° C. for two weeks, incubation at 56° C. for one hour, or Seitz filtration, did not result in an appreciable loss of potency of the serum.

Incubation of the serum specimens with cultures of *M. tuberculosis* H37Rv, *Mycobacterium sp.* ATCC 607, or with D-29 phage at 37° C. for one or two days caused no loss of the antiphage propagation effect when the filtered serum was later tested for this property.

Effect of Serum on the Survival of D-29 Phage

In several experiments it was found that there was no loss of titer of D-29 phage from the initial concentrations of approximately 2×10^7 plaques per ml. when incubated with or without 80 per cent serum at intervals of four and eighteen hours.

DISCUSSION

These studies have revealed a substance in human serum which inhibits the propagation of D-29 mycobacteriophage with strains of human tubercle bacilli, but not with the saprophytic *Mycobacterium* ATCC 607. The factor is heat stable and persists for days or weeks in serum stored at room or refrigerator temperatures. It is precipitated in the gamma globulin fraction of serum by ammonium sulfate, and is inactivated by treatment with trypsin. These tests strongly suggest that the active component is protein in nature, probably a gamma globulin. Its presence in about the same titer in sera from tuberculin-negative and tuberculin-positive donors, and from patients with active tuberculosis, suggests that it is not an antibody and may be a normal constituent of serum. The fact that it is relatively heat stable differentiates it from complement and properdin.

Some evidence concerning the site of action was gained from the foregoing studies. The failure of serum to inactivate phage directly, or to prevent its propagation on strain ATCC 607, indicates that its site of action is on the tubercle bacilli. Since it was found that adsorption of phage on tubercle bacilli was not prevented by serum (in fact, adsorption in serum was significantly greater than that observed in a broth control), the tubercle bacilli were apparently altered in such a manner that propagation was prevented because of defective adsorption or interference at some later stage of propagation.

Several investigators have described an essentially similar effect of serum on the lysis of other bacterial genera by bacteriophage. In 1921,

Gratia and Jaumain (3) found that serum in dilutions as great as 1 to 500 inhibited lysis by phage of colon bacilli and staphylococci. They found that the factor remained active after heating the serum at 70° C. Their studies also revealed that failure of lysis was associated with failure of increase in plaque-forming units. Other investigators have described inhibition of lysis of colon bacilli, dysentery bacilli, typhoid bacilli, streptococci, and staphylococci by their respective phages, variously in the presence of intestinal mucus and serum (4), pus (5), urine (6), and defibrinated blood (7). In 1932, Colvin (8) confirmed this effect of serum, working with streptococcal, staphylococcal, and anthrax phage-bacterial systems. In his studies he found that when serum-phage-bacterial mixtures were subcultured to the surface of agar, the serum was absorbed into the medium and lytic plaques developed. He reasoned that serum had not prevented adsorption of phage by the bacteria and that, when serum was removed from a loose combination with the bacterium by adsorption into agar, lysis was allowed to proceed.

Despite the differences in methods employed and the use of several different phage-bacterial systems, there is no conflict between the present studies and those referred to above. In a positive sense there is substantial agreement in the studies in which comparisons are possible. It thus seems probable that there is a gamma globulin in normal human serum, pus, and certain other tissue fluids which, through combination with the host bacterium, prevents phage propagation by some means other than interference with adsorption.

No explanation is evident at present for the failure of serum to interfere with the propagation of D-29 phage on the saprophytic *Mycobacterium* ATCC 607; however, it was established that incubation of serum with ATCC 607 did not remove this property of serum. It is tempting to consider that, since this organism is not pathogenic, it may react differently with serum than disease-producing organisms. There is some scanty support for this in the report of Gratia and Mutsaars (9), who found that serum did not inhibit lysis of *Staphylococcus albus*, an organism which is usually nonpathogenic, and the observation of Colvin (8) that lysis of *B. subtilis* was less interfered with by serum than several pathogenic species tested.

Summary

Fresh human serum contains a substance, probably a gamma globulin, which inhibits the propagation of D-29 mycobacteriophage on human tubercle bacilli, but not on the saprophytic *Mycobacterium* ATCC 607. It is present in about the same titer in serum from tuberculin-negative and tuberculin-positive donors and in serum from patients with active tuberculosis. The factor persists after heating for one hour at 56° C., but is inactivated by trypsin.

Adsorption of D-29 phage to tubercle bacilli is greater in serum than in its absence. Since it was shown that phage survives exposure to serum, and propagation is not prevented with strain ATCC 607, it appears that serum causes a reversible, abnormal adsorption or interferes at some later stage of propagation by a direct influence on the tubercle bacilli.

Sumario

Un Factor del Suero que Impide la Propagación del Micobacteriófago D-29 con Bacilos Tuberculosos Humanos

El suero humano reciente contiene una substancia, probablemente una globulina gamma, que impide la propagación del micobacteriófago D-29 en bacilos tuberculosos humanos, pero no en el saprofito *Mycobacterium* ATCC 607. Se halla presente aproximadamente al mismo título en el suero de los donantes negativos y positivos a la tuberculina y en el suero de los enfermos que tienen tuberculosis activa. El factor persiste después de la calefacción por una hora a 56° C. y es inactivado por la tripsina.

La adsorción del bacteriófago D-29 a los bacilos tuberculosos es mayor en el suero que a falta de éste. Como se demostró que el bacteriófago sobrevive la exposición al suero, y que no impide su propagación la cepa ATCC 607, parece que el suero provoca una adsorción anormal reversible o que interviene en alguna etapa más tardía de propagación por su influjo directo sobre los bacilos tuberculosos.

Resume

Un facteur du sérum qui prévient la multiplication du mycobactériophage D-29 sur les bacilles tuberculeux humains

Le sérum humain frais contient une substance, probablement une gamma globuline, qui inhibe la multiplication du mycobactériophage D-29 sur les bacilles tuberculeux humains, mais non sur le *Mycobacterium* ATCC 607. Il est présent

environ au même titre dans le sérum provenant de donneurs à réaction positive ou à réaction négative à la tuberculine, et dans le sérum des malades atteints d'une tuberculose en activité. Ce facteur persiste après chauffage à 56° C pendant une heure, mais il est inactivé par la trypsine.

L'adsorption du phage D-29 aux bacilles tuberculeux est plus grande dans le sérum qu'en l'absence de sérum. Etant donné qu'il a été démontré que le phage survit à l'exposition au sérum et que la multiplication n'est pas prévenue avec la souche ATCC 607, il semble que le sérum cause une adsorption anormale réversible ou qu'il intervient à un stade ultérieur de la multiplication par une influence directe sur les bacilles tuberculeux.

Acknowledgment

We wish to acknowledge the able technical assistance of Miss Frances Foster in this work.

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THE IN VITRO EFFECT OF SPECIFIC AND NONSPECIFIC BACTERIAL PRODUCTS ON THE MIGRATION OF LEUKOCYTES FROM TUBERCULIN-SENSITIZED GUINEA PIGS[1,2]

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(Received for publication December 8, 1958)

INTRODUCTION

In early experimentation on delayed hypersensitivity in tissue cultures, Rich and Lewis (1, 2) demonstrated a specific cytotoxic effect of tuberculin upon macrophages from the spleen of tuberculin-sensitized animals. Since then, many workers have studied *in vitro* the hypersensitivity of different kinds of cells from the tuberculous host. The majority have found that polymorphonuclear leukocytes, macrophages, and fibroblasts from tuberculin-sensitized animals were specifically sensitive to tuberculin (3–13).

A specific inhibition by tuberculin of the migration of leukocytes derived from tuberculous patients and tuberculin-positive healthy subjects has been reported by O'Neill and Favour (11), among others. Hall and Scherago (13) used guinea pigs in some of their experiments. They noted that the specific cytotoxic effect of tuberculin was obtained more regularly and to a higher extent with leukocytes from tuberculous laboratory animals than with human cells.

On the other hand, it has been observed that the tuberculous body also reacts hyperergically to various nontuberculous stimuli, as, for example, culture filtrates of *E. coli* or meningococci (14, 15). Freund (15) demonstrated that a parenteral injection of coli filtrate causes hemorrhage in tuberculous lesions in infected guinea pigs in a manner similar to tuberculin. Bordet (14) and Rich (16) discussed the possibility that the basis of this nonspecific hyperergy might be a Shwartzman phenomenon. It has also been shown that the site of a tuberculin reaction in the skin reacts hyperergically to such nonspecific stimuli as culture filtrates of *E. coli* with intravenous administration as well as when injected directly into the site of the tuberculin reaction in the skin (17–19).

This nonspecific reactivity of the tissues of tuberculin-sensitized animals has been recently demonstrated *in vitro* in a study on fibroblast and epithelial-cell cultures (20). The growth of the cells was shown to be inhibited by tuberculin as well as by *E. coli* culture filtrates.

In the present series of experiments, typical endotoxin lipopolysaccharides from two species of gram-negative bacteria and a pneumococcal polysaccharide, in addition to culture filtrates of *E. coli*, were selected for a study of their cytotoxic effect upon leukocytes from tuberculin-sensitized animals. The various preparations were compared with Old Tuberculin for their inhibitory *in vitro* action on the migration of leukocytes.

MATERIALS AND METHODS

Leukocyte Donors

Male guinea pigs weighing 400 to 600 gm. were sensitized by BCG[3] vaccination and subsequent injections of killed bovine tubercle bacilli (strain K 3) emulsified in paraffin to a concentration of 10 mg. per ml.[4] The BCG dosage was 1 ml. intraperitoneally and 0.5 ml. subcutaneously of a 20-mg. per ml. stock suspension. A dose of 0.1 or 0.2 ml. of the K 3 vaccine was injected subcutaneously five to seven times at one-week intervals. The tuberculin sensitivity of the animals was controlled about every third month by an intracutaneous injection of 0.1 ml. of standard Old Tuberculin (OT) diluted 1:100. If the tuberculin skin reaction was weaker than about 20 × 20 mm., the animals were given a booster dose of 0.2 ml. of K 3 vaccine subcutaneously.

Untreated male guinea pigs weighing 400 to 600 gm. and reacting negatively to OT were used as controls.

Preparations

Old Tuberculin: Two lots of bovine tuberculin without preservatives[4] were used. The final dilution employed was 1:100; in some experiments it was 1:1,000. Sterile Sauton medium, the medium used for the preparation of OT, served as a control.

[1] From the Central Bacteriological Laboratory of Stockholm City, Stockholm, Sweden.

[2] This investigation was supported by grants from the Swedish National Association Against Tuberculosis and Other Social Diseases.

[3] Kindly made available by Dr. O. Sievers, Gothenburg Bacteriological Laboratory, Gothenburg.

[4] Kindly made available by Dr. L. Silverstolpe, the State Bacteriological Laboratory, Stockholm.

Coli filtrate: Six-to-eight-day-old tryptic digest broth cultures of *E. coli* 6038 (from a case of pyelitis) were filtered through a Seitz E. K. filter. The filtrate was controlled to possess preparative activity in the Shwartzman phenomenon.

Lipopolysaccharides of E. coli and Serr. marcescens: Two preparations of *E. coli* 3650 (from a case of cystopyelitis), Coli 6 and Coli 9, and one preparation of *Serr. marcescens* S 1,[5] were used.

Cultures for the preparation of Coli 6 and S1 were grown in a tryptic digest broth. For the preparation of Coli 9 a synthetic medium with glucose as carbon source was used.[6] The bacterial cells were separated from the culture medium by means of a Sharples centrifuge. The sedimented cells were extracted with a phenol-water mixture according to the procedure described by Westphal and associates (21).[7] A solution was obtained which contained the lipopolysaccharides of the bacteria, but which also had large amounts of nucleic acids. The lipopolysaccharides were separated from the nucleic acids either by precipitation with alcohol (21) or by centrifugation in a preparative ultracentrifuge (22). The batch of Coli 6 was treated in the former way; the batches of Coli 9 and S 1 were treated with the latter method. The centrifugation were carried out in a Spinco model L ultracentrifuge using a No. 30 rotor. The centrifugation speed was 30,000 r.p.m.; the centrifugation time, thirty minutes. The sedimented lipopolysaccharide material was resuspended in distilled water and washed three times by centrifugation at 30,000 r.p.m.

The lipopolysaccharides were tested in rabbits for preparatory activity in the Shwartzman phenomenon. They were still active in a dilution of 1:12,500. The dry weights and protein impurities of the different batches were as follows:

	Dry Weight *Per Cent*	Protein Content *(Per Cent of Dry Substance)*
Coli 6	0.44	5.9
Coli 9	0.24	0.12
S 1	0.21	0.25

Figure 1 is a paper chromatogram of Coli 9 preparation.

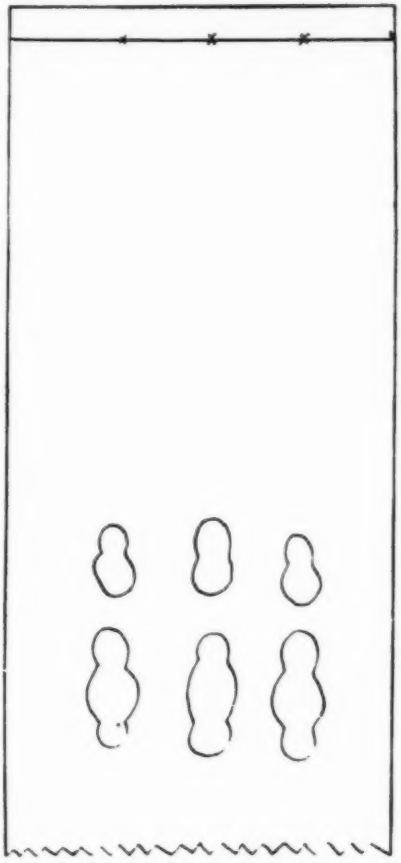

FIG. 1. Paper chromatogram of hydrolyzed lipopolysaccharides (Coli 9). Hydrolysis was performed with 2 N H_2SO_4 and took place for two hours at 100° C. The chromatographic pattern in the middle of the figure represents the hydrolyzed polysaccharides. The patterns to the left and right were obtained by applying a mixture of mannose, glucose, galactose, glucosamine, and galactosamine to the paper.

Pneumococcal polysaccharide: A highly purified polysaccharide from pneumococcus type 2[8] was used. The dry weight of the stock solution employed was 0.20 per cent.

The starting dilutions of the preparations used in the experiments were determined by titrating

[5] The *Serr. marcescens* S1 strain was generously supplied by the courtesy of Dr. M. Y. Shear, National Institutes of Health, Bethesda, Maryland.

[6] The bacteria used for the preparation of Coli 9 were grown in mass culture at the Bacteriological Department, Karolinska Institutet, Stockholm, by the courtesy of Dr. C.-G. Hedén.

[7] Dr. C. Weibull from the biochemical department of this laboratory has kindly prepared and analyzed all three lipopolysaccharide batches.

[8] Kindly made available by Dr. B. Björklund of the State Bacteriological Laboratory, Stockholm.

the maximal dose that did not appreciably inhibit the migration of leukocytes from tuberculin-negative animals.

Method of Experimentation

The determination of the hypersensitivity of the leukocytes was based on measurements of inhibition of their migration in the presence of different substances. The method used was a modification of that described by O'Neill and Favour (11). Three series of experiments provide the data for this report. In the first series, the migration of leukocytes from tuberculin-sensitized and normal animals was studied in the presence of coli filtrate; in the second series, in the presence of the batch of Coli 6; and in the third series, in the presence of the batches of Coli 9 or S 1 lipopolysaccharides, or pneumococcal polysaccharide. In each experimental series the migration of leukocytes was also tested in the presence of Old Tuberculin.

The capillary-tube "cultures" were prepared in the following way: All glassware, with the exception of capillaries, was coated with silicone. The capillary tubes were drawn from Pyrex® glass and had an internal diameter of about 1 mm. The animals were bled through heart puncture into tubes containing heparin to a final concentration of 0.1 mg. per ml. of blood. The tubes were cautiously shaken and the preparation to be tested was added in the concentration desired. The dilution medium was bovine amniotic fluid. The capillary tubes were filled to two-thirds of their length, and one end was heat-sealed. Seven to ten capillaries were prepared for every concentration. Capillary tubes containing only blood and bovine amniotic fluid served as controls. The capillary tubes were centrifuged at 1,500 r.p.m. for fifteen minutes and incubated overnight at 39°C. in a vertical position. They were examined the following day. The extent of leukocytic migration, that is, the distance from the buffy coat to the

TABLE 1

CYTOTOXIC INDICES OF OLD TUBERCULIN AND E. COLI FILTRATE FOR MIGRATION OF LEUKOCYTES FROM NORMAL AND TUBERCULIN-SENSITIZED GUINEA PIGS

Animal	Date	Control Migration	Old Tuberculin		Coli Filtrate		Sauton Medium		Tryptic Broth	
			1:100	1:1,000	1:50	1:100	1:100	1:1,000	1:50	1:100
	(*1956*)	(*mm.*)								
Normal Animals										
206	4–23	0.8	1.00	1.00			0.88	0.88		
211	4–26	1.7	0.76	0.76			0.94	1.10		
14	5–14	2.2	0.90	0.81		0.96	0.96	0.81		0.90
32	5–18	1.8	0.61	0.66	1.00	1.10	0.94	0.84	1.30	1.00
31	5–23	1.0		1.00		0.90	1.00	1.00	1.00	1.00
211	5–28	2.1	0.95	0.95	0.76	0.95	0.95	1.00	0.86	1.00
14	5–29	2.8	1.00	0.93	0.50	0.89	1.00	0.93	0.96	0.93
211	6–8	1.6	0.87	1.00	0.93	1.10	1.20	1.20	0.93	1.00
204	6–12	1.5	0.86	0.80	0.93	0.93	0.86	0.93	0.80	0.80
31	6–17	0.7	1.10	1.30	1.40	1.40	1.40	1.30	1.30	1.40
208	6–18	1.9	0.94	0.94	0.84	0.89	0.89	0.89	0.84	0.81
184	6–20	0.9	0.66	1.00	0.88	1.00	1.00	1.10	0.77	0.90
Sensitized Animals										
149	4–23	1.8	0.44	0.39			1.00	1.00		
170	4–26	1.8	0.17	0.44			1.00	1.00		
170	5–14	1.5	0.26	0.60		0.80	0.80	1.00		0.80
229	5–18	0.8	0.50	0.75	0.63	0.63	1.10	1.10	0.75	0.63
235	5–23	1.3	0.61	0.61	0.61	0.61	1.10	1.10	1.10	1.00
166	5–28	2.6	0.65	1.00	0.69	0.61	1.00	1.00	0.96	1.00
234	5–29	1.5	0.33	0.53	0.40	0.46	1.00	1.00	0.73	0.80
164	6–8	2.1	0.48	0.86	0.76	0.90	1.20	1.20	0.95	1.10
144	6–12	2.1	0.57	0.66	0.53	0.62	0.90	0.95	0.86	0.90
154	6–15	1.3	0.46	0.92	0.56	0.46	0.92	1.00	0.75	0.92
234	6–18	2.6	0.38	0.53	0.53	0.65	0.80	0.88	0.77	0.88
144	6–20	2.1	0.48	0.53	0.53	0.57	0.81	0.86	0.62	1.00

upper borderline of migrating leukocytes, was measured under low-power magnification by means of an ocular micrometer. The degree of inhibition was expressed as the ratio between the migration values obtained with and without preparation added to the bovine amniotic fluid. This ratio was termed cytotoxic index.

TABLE 2

CYTOTOXIC INDICES OF OLD TUBERCULIN AND COLI 6 LIPOPOLYSACCHARIDES FOR MIGRATION OF LEUKOCYTES FROM NORMAL AND TUBERCULIN-SENSITIZED GUINEA PIGS

Animal	Date	Control Migration	Old Tuberculin		Coli 6		Sauton Medium	
			1:100	1:1,000	1:50	1:100	1:100	1:1,000
	(1956)	(mm.)						
Normal Animals								
30	8-7	2.0				1.50		
37	8-7	2.4				1.04		
43	8-10	1 6			0.88	0.88		
208	8-14	1.0			1.00	1.00		
43	8-24	1.1	1.00		0.90	1.09	0.90	
30	8-27	2.6	0.86		0.86	0.86	0.86	
184	9-6	1.6	0.87	1.00	0.81	0.87	1.06	1.00
211	9-6	2.1	0.86	1.00	1.00	1.05	0.98	1.00
207	9-7	1.8	1.00	1.05	1.00	1.00	1.00	1.00
44	9-7	2.2	0.86	0.95	0.95	0.86	0.86	0.86
206	9-19	1.5	1.00	1.00	0.93	1.06	1.00	1.00
39	9-29	3.8	1.00	0.97	0.95	0.95	1.05	1.02
16	10-3	1.4	0.86		0.78			
17	10-3	2.0	0.81		0.90			
21	10-3	1.4	0.86		0.93			
Sensitized Animals								
229	8-10	2.1			0.76	0.76		
242	8-20	1.2	0.42		0.66	0.92	0.92	
244	8-20	1.6	0.25		0.50	0.50	0.81	
246	8-22	1.2	0.33		0.66	0.66	0.83	
247	8-22	1.5	0.86		1.13	1.13	0.86	
253	8-24	1.7	0.29		0.53	0.53	0.82	
264	8-14	1.7	0.22			0.59		
267	8-14	1.0	0.50			1.00		
277	8-29	1.0	0.30		0.40	0.40	0.90	
253	8-31	1.3	0.30		0.38	0.46	0.84	
157	9-5	1.9	0.26		0.42	0.42	0.84	
229	9-5	1.8	0.33		0.55	0.72	0.88	
215	9-10	1.8	0.38	0.55	0.50	0.55	1.05	1.05
222	9-14	2.0	0.60	0.80	0.80	0.85	0.95	0.90
223	9-14	2.0	0.50	0.80	0.75	0.85	1.05	1.05
225	9-14	2.0	0.45	0.75	0.45	0.55	1.00	1.05
140	9-21	2.1	0.43	0.66	0.43	0.48	1.05	1.05
220	9-26	1.7	0.47	1.06	0.53	0.76	1.17	1.11
254	9-26	1.9	0.56	0.79	0.74	0.95	1.53	1.53
250	9-29	2.6	0.50	0.69	0.62	0.69	0.88	
140	10-5	2.7	0.37	0.59	0.52	0.59		
160	10-5	2.6	0.50	0.85	0.65	0.65		
157	10-5	1.7	0.53	0.76	0.64	0.64		

RESULTS

All three series of experiments (tables 1–3) demonstrate clearly the cytotoxic effect of tuberculin on the leukocytes from tuberculin-sensitized animals. An increase of the tuberculin dilution from 1:100 to 1:1,000 did not abolish the cytotoxicity (table 1). The high index (close to 1.0) for the sterile Sauton medium control showed that the medium by itself has no inhibitory activity.

The *E. coli* culture filtrate and the purified lipopolysaccharides from *E. coli* and *Srer. marcescens*, diluted just enough so as not to cause any appreciable inhibition of the migration of leukocytes from tuberculin-negative donors, still had a clear inhibitory action on cells from tuberculin-sensitive animals. When the dilution was continued by more than one twofold step, the inhibitory activity was completely abolished. The sterile tryptic broth controls did not inhibit the migration of either type of cells (table 1).

On the other hand, the pneumococcal polysaccharide displayed, in concentrations not toxic for the normal cells, no difference in its activity on leukocytes from normal and tuberculin-sensitized animals (table 3). In table 4 the results of a statistical analysis of the experimental data are presented. It will be seen that the differences between the cytotoxic indices for tuberculin-positive and -negative animals are highly significant. A scrutiny of the tables shows that a number of guinea pigs have been examined two or more times at varying intervals. With some exceptions, their sensitivity to specific and nonspecific agents remained unchanged.

DISCUSSION

As to the cytotoxic effect of tuberculin upon leukocytes from tuberculin-sensitized animals, the present results confirm earlier observations. The specific inhibition of the migration of tuberculin-sensitized leukocytes was obtained quite regularly. This finding is in agreement with the observation of Hall and Scherago (13) that guinea pig cells display the hypersensitivity phenomenon more regularly than do human cells. However, in the present experiments, the major object was not to study the specific action of tuberculin particularly, but whether various nonspecific bacterial products of endotoxin nature have a similar

TABLE 3

CYTOTOXIC INDICES OF OLD TUBERCULIN, COLI 9 AND S 1 LIPOPOLYSACCHARIDES, AND PNEUMOCOCCAL POLYSACCHARIDE FOR MIGRATION OF LEUKOCYTES FROM NORMAL AND TUBERCULIN-SENSITIZED GUINEA PIGS

Animal	Date	Control Migration	Old Tuberculin		Coli 9	S 1		Pneumococcal Polysaccharide		
			1:100	1:1,000	1:400	1:100	1:200	1:25	1:50	1:100
	(1957)	(mm.)								
Normal Animals										
46	6–5	1.1	0.91	0.86		0.83				
47	6–13	1.1		1.01		1.00				
49	6–14	1.8		0.84	0.86	0.85				
50	6–14	1.1		0.94	0.98	0.79				
24	6–18	1.7	0.95		0.94	0.80	0.96			
37	6–18	1.1	1.00		0.82	0.94	0.95			
207	6–20	1.4	1.12		1.04	0.96	0.99			
24	6–24	1.2	0.95		0.98	0.96	0.96			
37	8–2	2.0	0.98		0.89	0.92	0.98			
59	8–2	1.2	0.98		1.03	0.93	0.94			
24	8–9	1.9	0.82		0.84	0.83	0.83			
22	8–9	1.7	1.05		1.02	0.92	1.01			
58	8–15	1.5	0.96				0.98	0.54	0.88	0.93
59	8–15	1.7	0.98				0.96	0.33	0.75	0.92
56	8–15	1.8						0.53	0.89	
24	8–16	2.2	1.12				1.02		0.70	0.91
207	8–23	1.6	0.95				1.01		0.81	1.03
55	9–3	1.3	0.81				0.82		0.77	0.86
58	9–3	1.7	0.97				1.07		1.04	1.07
88	11–6	1.5						0.52		
84	11–6	1.7						0.75		
83	11–12	1.9						0.42		
96	11–12	2.8						0.58		
46	11–18	1.6						0.36		
59	11–18	2.0						0.57		
Sensitized Animals										
223	6–5	1.5	0.33	0.39		0.47			0.91	0.97
279	6–6	1.7		0.46		0.33				
281	6–13	0.9		0.58		0.34				
286	6–14	1.7		0.30	0.26	0.36				
289	6–18	0.9	0.48		0.59	0.33	0.28			
296	6–20	1.8	0.49		0.66	0.26	0.37			
298	6–20	2.0	0.32		0.41	0.26	0.31			
295	6–20	1.9	0.51		0.55	0.36	0.32			
299	6–24	1.6	0.57		0.48	0.47	0.42			
313	7–29	1.3	0.55		0.61	0.46	0.52			
220	8–5	1.8	0.46		0.64	0.44	0.40			
317	8–5	1.7	0.59		0.53	0.35	0.39			
279	8–9	1.6	0.48		1.01	0.87	0.96			
223	8–23	1.6	0.89				0.87			
279	8–23	1.5	0.38				1.01		1.08	1.04
281	8–23	1.8	0.56				0.52		0.85	1.09
283	9–3	2.1	0.35				0.34		0.79	0.96
284	9–3	2.2	0.55				0.37		0.83	0.88
300	9–6	2.4	0.34		0.46		0.60		0.71	0.93
295	9–6	2.0	0.76		0.71		0.31		1.08	0.98
298	9–6	1.8	0.44		0.36		0.50		0.97	0.98
220	9–10	2.1	0.40		0.56		0.42		0.66	0.76
317	9–10	2.5			0.59		0.59		0.84	0.92
289	9–10	1.8	0.66		0.53		0.54		0.90	1.04

TABLE 4

PROBABILITY VALUES FOR THE CYTOTOXIC EFFECT OF SPECIFIC AND NONSPECIFIC BACTERIAL PRODUCTS ON THE MIGRATION OF LEUKOCYTES

Antigen	Concentration	N_1	N_2	$\bar{X}_1$	$\bar{X}_2$	t	P
Old Tuberculin	1:100	37	53	0.92	0.46	16.01	<0.001
	1:1,000	22	27	0.95	0.65	6.33	<0.001
E. coli filtrate	1:50	8	9	0.90	0.58	3.48	<0.01 >0.001
	1:100	10	10	1.01	0.63	5.82	<0.001
Coli 6	1:50	13	21	0.91	0.60	6.05	<0.001
	1:100	12	23	1.01	0.68	4.89	<0.001
Coli 9	1:400	10	16	0.94	0.56	6.69	<0.001
S 1	1:100	12	13	0.89	0.41	9.75	<0.001
	1:200	14	20	0.96	0.50	7.72	<0.001
Pneumococcal polysaccharide	1:50	7	11	0.83	0.87	0.65	<0.6 >0.5
Sauton medium	1:100	20	29	0.99	0.97	0.48	<0.7 >0.6
	1:1,000	18	19	0.99	1.04	1.12	<0.3 >0.2
Tryptic broth	1:50	9	9	0.97	0.83	1.72	<0.2 >0.1
	1:100	10	10	0.97	0.90	1.35	<0.4 >0.3

N_1 = number of experiments with leukocytes from normal animals
N_2 = number of experiments with leukocytes from tuberculin-sensitized animals
$\bar{X}_1$ = mean cytotoxic index for experiments with leukocytes from normal animals
$\bar{X}_2$ = mean cytotoxic index for experiments with leukocytes from tuberculin-sensitized animals
Probability values calculated according to Student's *t*-test

$$t = (\bar{X}_1 - \bar{X}_2) \sqrt{\frac{(N_1 + N_2 - 2)\ N_1\ N_2}{N_1 + N_2\ [S\ (X_1 - \bar{X}_1)^2 + S\ (X_2 - \bar{X}_2)^2]}}.$$

cytotoxic effect. It was thus hoped to establish an analogy to the hypersensitivity in tuberculosis to nonspecific stimuli previously described (14, 15, 18, 19). The present results do suggest such an analogy.

The enhancement of reactivity of cells in tuberculosis presents several problems for discussion. The inhibition of migration in the presence of nonspecific bacterial products may be only an expression of a general vulnerability of the sensitized cells to a number of nonspecific injuries, a vulnerability independent of the specific tuberculin reactivity. On the other hand, bacterial endotoxins and tuberculin may have some common denominator for their effect on leukocytes from tuberculin-sensitized animals. The endotoxins are lipopolysaccharides, whereas the active principle of tuberculin is considered to be of protein nature.

It must be admitted that at present a perplexing collection of data has been assembled concerning the hypersensitivity of leukocytes from tuberculin-sensitized animals. For, these cells seemingly react to such diverse agents as tuberculoproteins and nontuberculous lipopolysaccharides of endotoxin nature. Certainly, a great deal more information is needed concerning the factors determining the specific tuberculin hypersensitivity and the exact nature of the eliciting agents before it will be possible to answer the question of why tuberculin-sensitized cells display such variation in their reactivity.

SUMMARY

Old Tuberculin and certain nonspecific bacterial products were tested for their inhibitory action *in vitro* upon the migration of leukocytes from tuberculin-sensitized guinea pigs. Cytotoxicity was displayed by Old Tuberculin, as well as by filtrates of *E. coli* cultures and lipopolysaccharides of *E. coli* and *Serr. marcescens*, but not by pneumococcal polysaccharide.

SUMARIO

El Efecto in Vitro de los Productos Bacterianos Específicos y Anespecíficos sobre la Migración de los Leucocitos de Cobayos Sensibilizados a la Tuberculina

Se comprobaron la tuberculina antigua y ciertos productos bacterianos anespecíficos en cuanto a su acción inhibidora *in vitro* sobre la migración de los leucocitos procedentes de cobayos sensibilizados a la tuberculina. Desplegó citotoxicidad la tuberculina antigua e hicieron otro tanto los filtrados de cultivos de la *E. coli* y los lipopolisacáridos de la *E. coli* y la *Serr. marcescens*, pero no el polisacárido neumocócico.

RESUME

Effet in vitro des produits bactériens spécifiques et non spécifiques sur la migration des leucocytes de cobayes sensibilisés à la tuberculine

La tuberculine de Koch et certains produits bactériens non spécifiques furent testés pour leur action inhibitrice *in vitro* sur la migration des leucocytes provenant de cobayes sensibilisés à la tuberculine. La cytotoxicité était révélée par la tuberculine de Koch ainsi que par les filtrats de cultures de *E. coli*, les lipopolysaccharides de *E. coli* et de *Serr. marcescens*, mais non par les polysaccharides pneumococciques.

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THE INHIBITION OF ISONIAZID INACTIVATION BY MEANS OF PAS AND BENZOYL-PAS IN MAN[1]

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(Received for publication July 16, 1958)

INTRODUCTION

In man, isoniazid is converted to acetyl-isoniazid, isonicotinic acid, and isonicotinuric acid, and these derivatives are then excreted in conjunction with the unchanged compound (1, 2). *In vitro*, the isoniazid derivatives have no, or only a very slight, antituberculous effect (3). The extent to which isoniazid is broken down to the above-mentioned compounds in the human body varies from one individual to another, but it seems to be quite constant over a certain length of time for one and the same person (1, 4). In patients who metabolize isoniazid quickly, the plasma concentration of free, bacteriostatically active isoniazid is relatively low. A relationship between the isoniazid metabolism and the success of isoniazid therapy has been postulated by Mitchell and Bell (5), as well as by Vivien and associates (6).

By means of enzymic tests *in vitro* and in the rabbit, Johnson and Corte (7, 8) have shown that various aromatic amines, particularly PAS, inhibit the *acetylation* of isoniazid. In man, several investigators have found a higher concentration of free isoniazid in the plasma, if isoniazid was administered in association with PAS instead of alone (9–12). In these studies, microbiologic methods of isoniazid determination have been employed. Making use of a chemical-colorimetric method, the present writers have also found higher values for free isoniazid in the plasma and in the urine on adding PAS (13).

In contrast to these findings, Peters (14), employing a chemical method of isoniazid determination in four subjects, was able to demonstrate no changes of free isoniazid concentration in the urine, regardless of whether isoniazid was administered with or without PAS.

For the tests described in the present studies, an improved method of determination for free isoniazid has been used, in contrast to those published earlier (13). Moreover, the totality of isoniazid and the isoniazid metabolites has been estimated, i.e., isoniazid, acetyl-isoniazid, isonicotinic acid, isonicotinuric acid, di-isonicotinoyl hydrazine (called hereafter "total isonicotinic acid derivatives"). On calculating the ratio of free isoniazid to total isonicotinic acid derivatives, the isoniazid metabolism can be estimated more adequately than on determining isoniazid alone, for this ratio depends less upon the rate of absorption. In the individual patient this ratio remains relatively constant over a period of several days, this being a requirement for the assessment of possible inhibitors of inactivation such as PAS.

Apart from PAS, benzoyl-PAS has also been examined with reference to the inhibition of isoniazid inactivation. In combination with isoniazid, benzoyl-PAS displays therapeutic and resistance-delaying effects similar to PAS (15, 16). In contrast to this latter, the calcium salt of benzoyl-PAS[2] is better tolerated with respect to the digestive system (16, 17).

METHODS

The investigations were carried out on male patients with pulmonary tuberculosis who showed no signs of hepatic or kidney involvement. All antituberculous treatment, as well as the administration of analgesics or hypnotics, was discontinued forty-eight hours prior to, as well as during, the trials. To elucidate the conditions of current isoniazid medication, both isoniazid and the PAS preparations were given by mouth. To examine the constancy of the degree of individual inactivation, 12 patients were given single oral doses of 500 mg. of isoniazid. In the twenty-four-hour urine, both free isoniazid and "total isonicotinic acid derivatives" were estimated. The test was repeated three times at intervals of three to four days.

For examining the effect of PAS and of benzoyl-PAS on isoniazid inactivation, the patients were given single doses of 300 to 500 mg. of isoniazid alone. One week later, the same patients were given the same isoniazid dose, as well as one of the PAS preparations in one to three fractional doses distributed over the day (for preparations and dosages, *see* table 1). In two test series, blood was withdrawn each time three hours after the admin-

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[2] Benzapas®, Smith-Dorsey, Lincoln, Nebraska.

istration of 500 mg. of isoniazid, and the per cent of free isoniazid in the plasma was determined. Only with this relatively high dose is it possible to estimate isoniazid chemically with sufficient accuracy. In the urine, too, both free isoniazid and total isonicotinic acid derivatives were estimated, and the free isoniazid in per cent of the total isonicotinic acid derivatives was calculated. In tests *4* to *6* it was impossible for technical reasons to determine the total isonicotinic acid derivatives. For estimating the influence of benzoyl-PAS on the isoniazid metabolism, the absolute amount of free isoniazid excreted in the twenty-four-hour urine was used in these cases.

The ratio of free isoniazid when isoniazid was administered in combination and free isoniazid when isoniazid was administered alone $\left(\frac{\text{isoniazid combination}}{\text{isoniazid}}\right)$ was calculated each time. For statistical evaluation, the logarithm of this ratio for each patient and the arithmetic mean of these logarithms were calculated, since it is the logarithms which show an approximately normal distribution. The mean values of these logarithms differ from 0 if PAS or benzoyl-PAS influences the concentration of free isoniazid. The statistical significance of this deviation from 0 was checked with the *t*-test of Student.

For estimating the influence of the PAS preparations, the antilogarithm of the mean of log $\frac{\text{isoniazid combination}}{\text{isoniazid}}$ is employed.

Determination of Free Isoniazid

The free isoniazid was extracted from the plasma by means of isoamyl alcohol-ether, according to the method of Kelly and Poet (18), and estimated in comparison with the method of Scott (19) with the aid of the color reaction with 1-chloro-2:4-dinitrobenzene. This reagent is specific for isoniazid, and gives no color reaction with acetyl-isoniazid, isonicotinic acid, isonicotinuric acid, di-isonicotinoyl hydrazine, PAS, or acetyl-PAS. Following extraction from plasma or water according to Kelly and Poet (18), 85 to 88 per cent of the added isoniazid is recovered in the extraction fluid.[2] Thus, for establishing a standard curve, the standard isoniazid dilutions in plasma must be extracted exactly according to the same method.

Standard deviations for 0.25 mg. of isoniazid per 100 ml. = ± 4.0 per cent; for 0.05 mg. of isoniazid per 100 ml. = ± 8.0 per cent.
Analytic threshold = 0.005 mg. of isoniazid per 100 ml.

Procedure

In a glass-stoppered shaking bottle, 3.2 gm. of ammonium sulfate were weighed, and 5 ml. of *plasma* plus 0.5 ml. of 1 N NaOH plus 30 ml. of isoamyl alcohol–ether (1:4) were added. The mixture was shaken for thirty minutes on the shaking machine, and was then centrifuged. With the aid of a pipette, 20 ml. of the ether solution were withdrawn and were then shaken for three minutes with 1.3 ml. of 0.1 N HCl. One milliliter of the aqueous solution was transferred into a second test tube, and 1 ml. of 0.1 N alcoholic KOH plus 100 mg. of borax plus 8 ml. of 1-chloro-2:4-dinitrobenzene solution (1.25 per cent in absolute ethanol, always freshly prepared) were added. The tubes were placed in a boiling water bath for twenty minutes, which resulted in complete evaporation of the ethanol. The tubes were then cooled, 3 ml. of methanol were added to the residue, and the mixtures were shaken and filtered. The solutions were assayed colorimetrically in the Beckman spectrophotometer, model DU, in cups of 10 mm. light path, at 530 mμ. The cups had to be placed 3 mm. higher in the holder to avoid errors due to the small amount of liquid. The blank solution consisted of plasma obtained prior to the administration of isoniazid. For the estimation in the *urine*, 1 ml. of diluted urine (1:10 to 1:20) was mixed, without extraction, with borax and chlorodinitrobenzene solution, and the procedure described above was then followed. The dyestuff was extracted with 5 ml. of methanol. When working with chlorodinitrobenzene, the operators showed allergic skin reactions.

For this reason, the method of Maher and coworkers (20) for estimating the free isoniazid in the urine was subsequently employed (*see* tests of figure 3). The determination of isoniazid in the plasma according to this method yielded nonreproducible values.

Determination of the Total Isonicotinic Acid Derivatives

By oxidation with $KMnO_4$ or ceric sulfate (21, 4), isoniazid, acetyl-isoniazid, and isonicotinuric acid are converted to isonicotinic acid, and this is then estimated by means of cyanogen bromide reagent after Rubin and associates (21). Previously, the main parts of PAS, acetyl-PAS, and benzoyl-PAS must be eliminated by extraction with ether. The extraction involves a certain loss of isonicotinic acid derivatives. Whether no PAS derivatives, or 20 mg. of PAS, or the equivalent quantities of benzyol-PAS or acetyl-PAS are added, the loss amounts to:

	Mean of Three Tests (*per cent*)
Isoniazid	5.5
Acetyl-isoniazid	12.0
Isonicotinic acid	12.5
Isonicotinuric acid	6.0

In order to keep the error to a minimum, isonicotinic acid must be used for establishing the standard curve. Thus, the maximal possible error (for 50 per cent free isoniazid of the total isonicotinic acid derivatives) is plus 3.5 per cent.

[2] Checked for 0.25 mg. of isoniazid per 100 ml. and 0.05 mg. of isoniazid per 100 ml. without PAS and in the presence of 20 mg. of PAS per 100 ml.

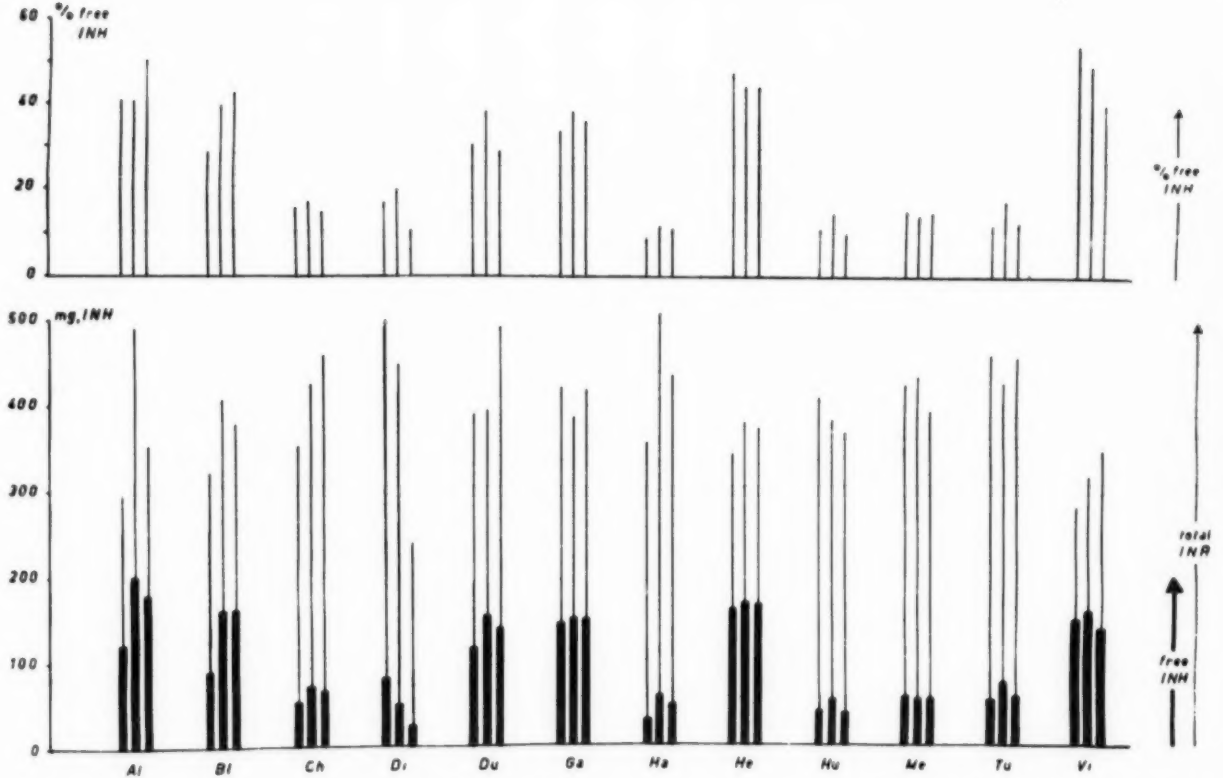

FIG. 1. Excretion of free isoniazid and total isonicotinic acid derivatives (in isoniazid equivalents), after oral administration of 500 mg. of isoniazid. Three tests, at intervals of three or four days, in 12 patients. (INH = isoniazid; INA = isonicotinic acid)

TABLE 1

RATIO OF FREE ISONIAZID IN PLASMA AND URINE FOLLOWING THE ADMINISTRATION OF AN ISONIAZID-PAS COMBINATION AND ISONIAZID ALONE

Test Number	Isoniazid Alone	Isoniazid with PAS or Benzoyl-PAS	Number of Patients	Ratio* of the Amounts of Free Isoniazid on Administration of an Isoniazid-PAS Combination to the Amounts on Administration of Isoniazid Alone				
				Plasma	Excretion			
	(mg.)			(3 hours)	(0–4 hours)	(4–10 hours)	(10–24 hours)	(0–24 hours)
1	500	500 mg. of isoniazid + 3 × 6.6 gm. of calcium PAS (= 3 × 5 gm. of PAS)	17	1.47†	1.47‡	1.66‡	2.62‡	1.67‡
2	500	500 mg. of isoniazid + 3 × 6.6 gm. of calcium B-PAS (= 3 × 5.4 gm. of B-PAS)	20	1.32‡	1.55‡	1.99‡	2.36†	1.59†
3	500	1,000 mg. of isoniazid-PAS (= 475 mg. of isoniazid + 525 mg. of PAS)	7		1.03	1.04	1.15	1.05
4	3 × 100	3 × 100 mg. of isoniazid + 3 × 4 gm. of calcium B-PAS	17					1.48†
5	3 × 100	3 × 100 mg. of isoniazid + 3 × 2 gm. of calcium B-PAS	17					0.91
6	2 × 250	2 × 250 mg. of isoniazid + 2 × 4 gm. of calcium B-PAS	12					1.73†

* Ratio = $\frac{\text{Free isoniazid (combined therapy)}}{\text{Free isoniazid (single therapy)}}$

The values marked † and ‡ differ significantly from 1.0: † $P < 0.03$; ‡ $P < 0.01$.

Standard deviation of the determination for 1.6 mg. per 100 ml. = ±2.4 per cent; for 0.2 mg. per 100 ml., = ±6 per cent.

Procedure

Two milliliters of *plasma* plus 2 ml. of distilled water plus 2 ml. of trichloracetic acid (10 per cent) were mixed in a centrifuge tube and the mixture was vigorously centrifuged. The clear supernatant fluid was poured into a glass-stoppered test tube, 8 ml. of ether were added, and the mixture was shaken for five minutes. After separation of the phases, the ether solution was completely sucked off and 4 ml. of the extracted aqueous solution were introduced into a graduated test tube. One milliliter of 20 per cent sulfuric acid was added, and the tubes were placed in a boiling water bath for twenty minutes. To the hot solution, aqueous ceric sulfate solution (15 per cent in 0.5 N H_2SO_4) was added dropwise until a yellowish color appeared. The excess ceric sulfate was reduced by means of ascorbic acid (1 per cent). To the colorless solution, phenolphthalein and 4 N NaOH were added until a pink color appeared. Then 2 N HCl was added until the color vanished. Ammonia buffer[4] was added up to 10 ml. and the resulting precipitate was collected on a filter. Five milliliters of the clear, colorless filtrate were mixed with 1 ml. of aqueous BrCN solution (10 per cent), and the extinction of the resulting yellow dyestuff was estimated after three to six minutes at 437 mμ (blank solution: with plasma obtained prior to the administration of isoniazid).

Two milliliters of *urine* diluted 1:10 were introduced into a glass-stoppered test tube, 2 ml. of 0.1 N HCl plus 8 ml. of ether were added, and the mixture was shaken for five minutes. The ether was completely sucked off, and 2 ml. of the aqueous solution were introduced into a graduated test tube. To this were added 2 ml. of distilled water plus 1 ml. of H_2SO_4 (20 per cent), and the mixture was heated for twenty minutes in a boiling water bath. Subsequently, oxidation was achieved with ceric sulfate as for the plasma, and the above procedure was then followed.

[4] Dissolve 87 gm. of K_2HPO_4 and 107 gm. of NH_4Cl in about 400 ml. of water and add 6.7 ml. of concentrated ammonia (28 per cent) to make 500 ml.

RESULTS

Constancy of the degree of individual acetylation: The amounts of excreted free isoniazid and of the total isonicotinic acid derivatives after administration of 500 mg. of isoniazid by mouth are shown in figure 1. Three estimations within one week showed the percentage of free isoniazid in the urine to be relatively constant, while the variations of the absolute amount of free isoniazid are greater. However, the variations from one patient

to another are very marked, as has already been reported by many investigators. If the difference of the chronologic deviations is compared with that of the individual variations with the aid of the analysis of variance (F-test), a high degree of significance results ($P \ll 0.01$), which means that the individual deviations are very much greater than the chronologic variations. In view of the

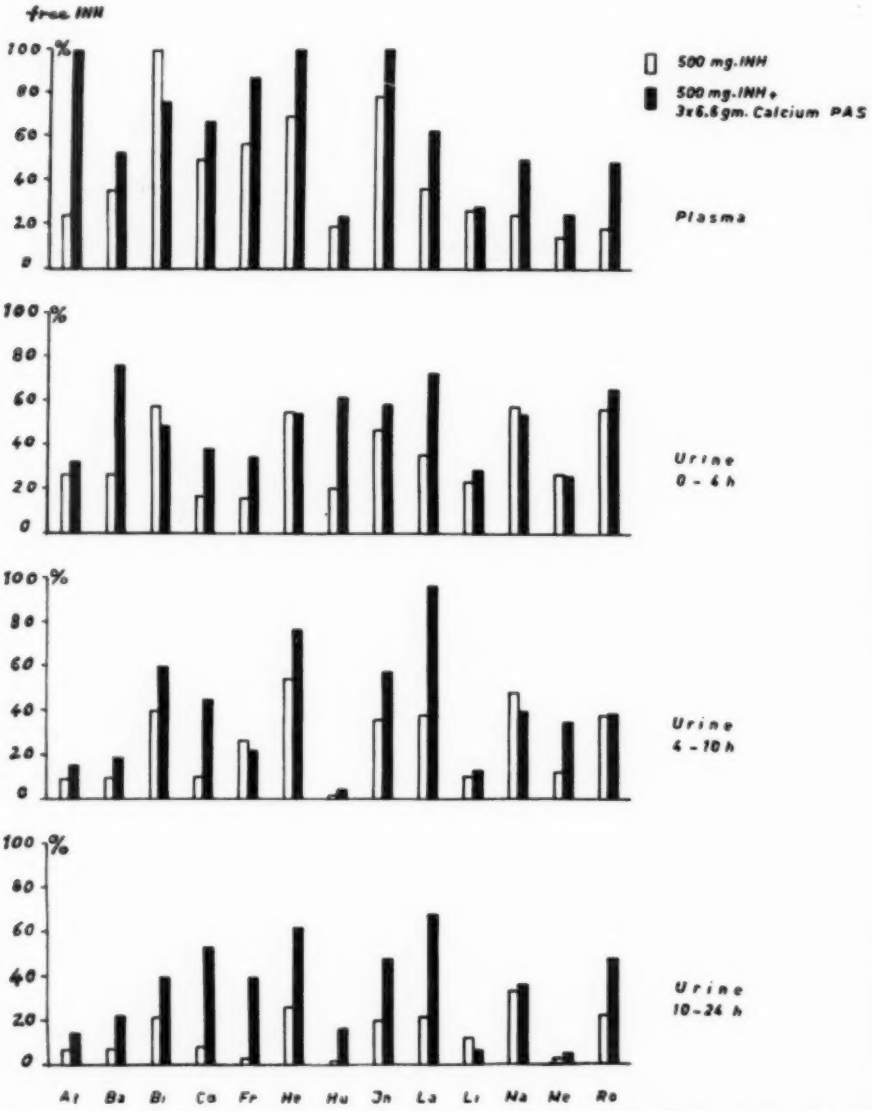

FIG. 2. Percentage ratio of the free isoniazid to total isonicotinic derivatives in the plasma (three hours after administration of isoniazid) and in the urine on administration of isoniazid alone, and isoniazid plus 3 × 6.6 gm. of calcium PAS (= 3 × 5 gm. of PAS) daily, in 13 patients. (INH = isoniazid)

constancy of the individual degree of inactivation, the effect of adding PAS or benzoyl-PAS to a constant isoniazid dose can be examined on different days by administering either product.

Effect of PAS and benzoyl-PAS on the isoniazid inactivation: It may be seen in table 1 that, on adding PAS (figure 2) or benzoyl-PAS (figure 3), a significant increase of the percentage content of

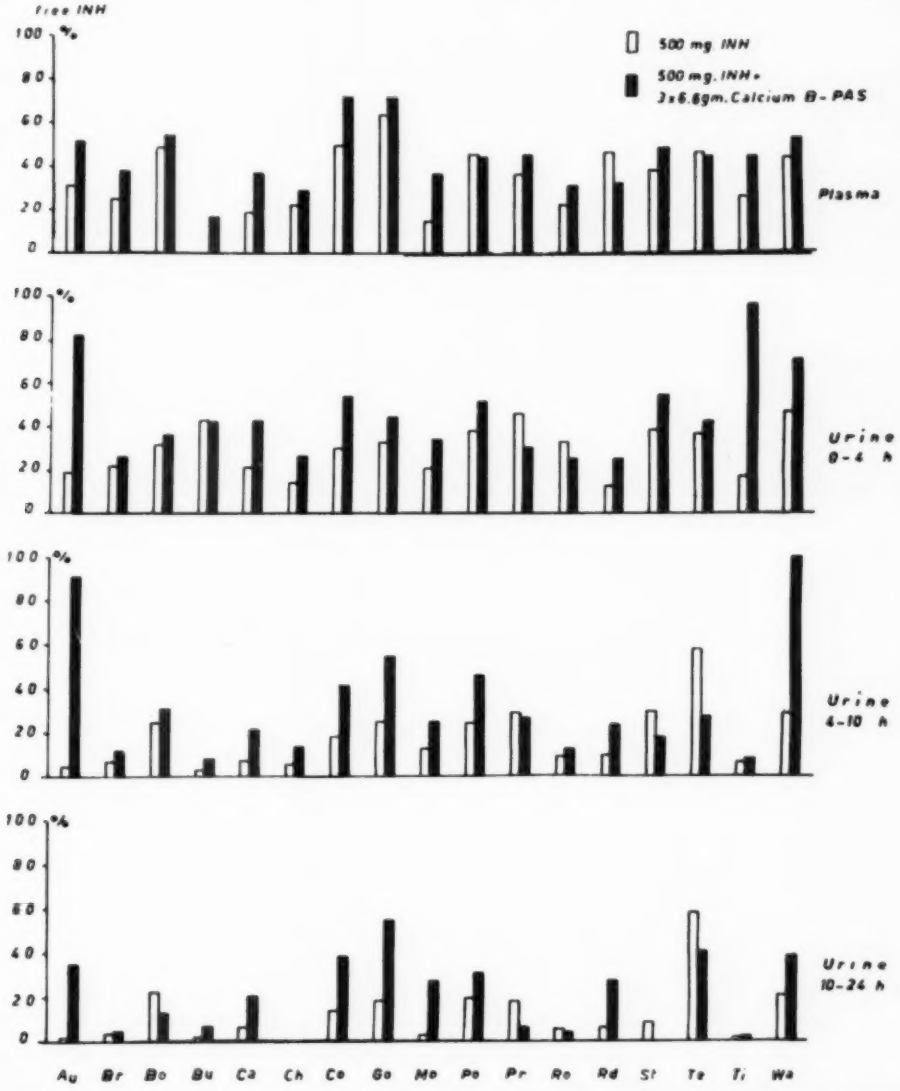

FIG. 3. Percentage ratio of the free isoniazid to total isonicotinic acid derivatives in the plasma (three hours after administration of isoniazid) and in the urine following administration of isoniazid alone and isoniazid plus 3 × 6.6 gm. of calcium benzoyl PAS (= 3 × 5.4 gm. of benzoyl-PAS) daily, in 17 patients. (INH = isoniazid)

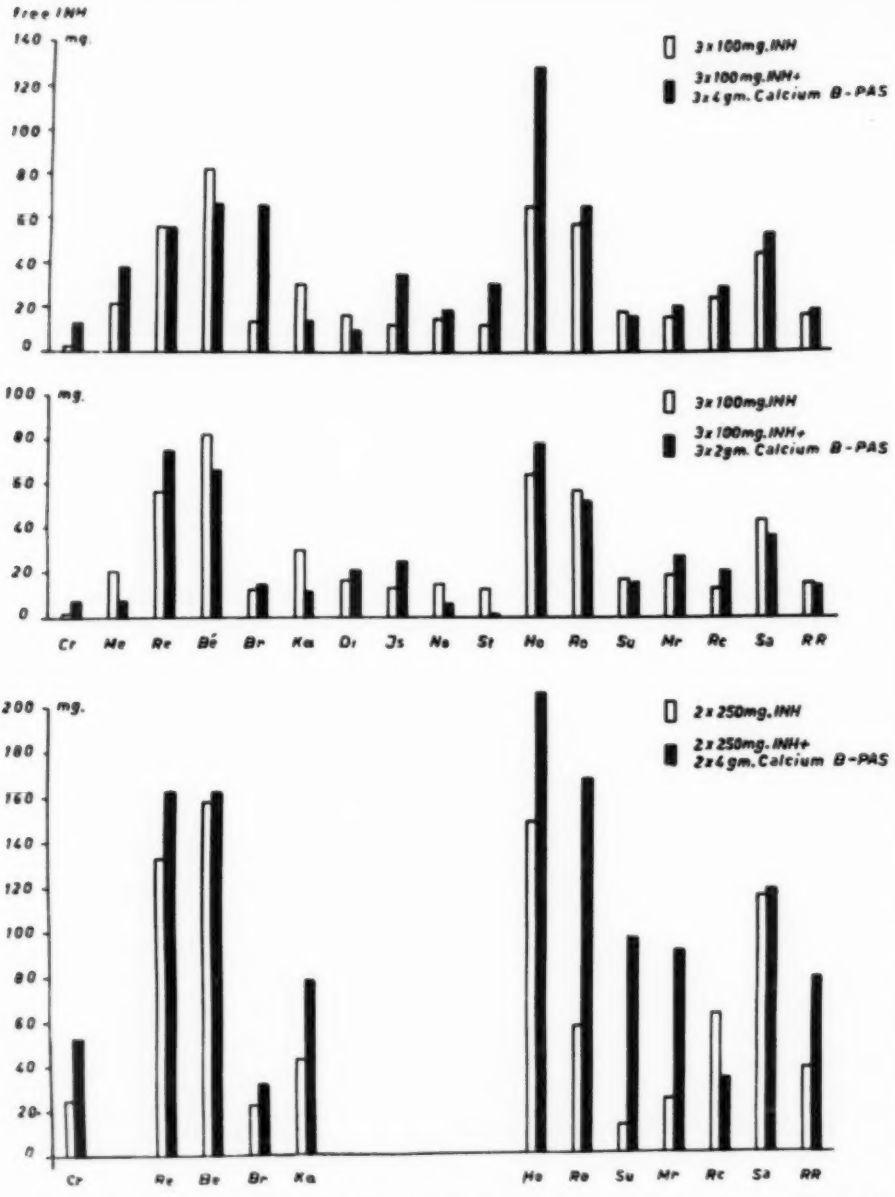

FIG. 4. Free isoniazid in the urine twenty-four hours after administration of either isoniazid or isoniazid plus 6, 8, or 12 gm. of calcium benzoyl-PAS per day (INH = isoniazid)

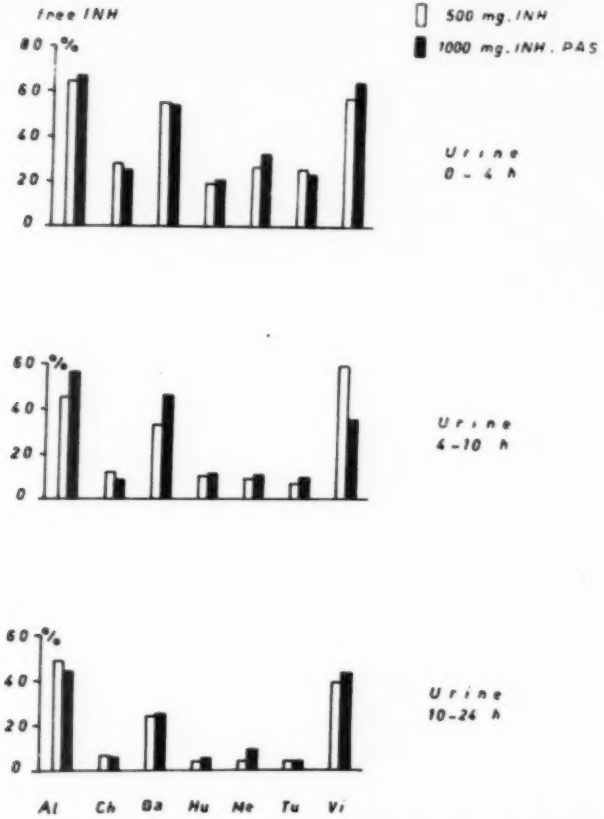

FIG. 5. Percentage ratio of free isoniazid to total isonicotinic acid derivatives in the urine following adminstration of 500 mg. of isoniazid or 1,000 mg. of the molecular isoniazid-PAS compound (containinig 475 mg. of isoniazid plus 525 mg. of PAS). (INH = isoniazid)

free isoniazid in the plasma and in the urine resulted. On the average, about 1.5 times more free isoniazid was found on giving 20 gm. of PAS daily in association with isoniazid than on administering isoniazid alone.

According to the plasma values, the influence of 20 gm. of calcium benzoyl-PAS seemed to be somewhat less marked than that of PAS, while according to the urine values it seemed to be a little more pronounced. However, the differences were not significant with a dosage of 6.6 gm. three times daily.

In the ten- and twenty-four-hour urine fractions, the effect of PAS and of benzoyl-PAS was greater because the proportion of free isoniazid, without administration of PAS or benzoyl-PAS, was usually very small. Calculated with reference to twenty-four-hour excretion, this is less important, however, because most of the isoniazid is excreted within ten hours.

For technical reasons, only free isoniazid could be determined in the urines in the tests involving small doses of benzoyl-PAS. The results of these determinations depend not only upon the spontaneous variations of the degree of inactivation, but also on the variations of absorption, so that the effect of benzoyl-PAS becomes less apparent. Nevertheless, the findings show clearly that only with doses of 4 gm. of calcium benzoyl-PAS twice daily and more can a depend-

able increase in free isoniazid be achieved (table 1, figure 4). Moreover, no significantly greater amounts of free isoniazid were found after the administration of the molecular isoniazid-PAS compound (para-aminosalicylate of isonicotinic acid hydrazide) than after isoniazid alone (figure 5).

Discussion

Examination of figure 1 shows that the excretion of the total isonicotinic acid derivatives reached 100 per cent of the administered dose in only about one-ninth of the cases, and that in several patients it amounted to only 60 per cent or less. This fact is attributed to incomplete absorption. A more thorough breakdown of isoniazid, involving the formation of analytically unrecoverable metabolites, is improbable. According to findings reported by Iwainsky and associates (22), absorption of isoniazid does not always seem to be complete. In the urine of normal subjects, these investigators recovered 45 to 96 per cent of the orally administered isoniazid dose in the form of metabolites, but they also found considerable amounts (up to 50 per cent) in the feces. From the variability of the absorbed amounts it appears that it is preferable to use the relative rather than the absolute concentrations of free isoniazid for the calculations.

Likewise, the fraction in percentage of free isoniazid does not remain entirely constant during the three test days, e.g., patients *Al*, *Bl*, *Du*, *Vi*, in figure 1. Thus, if it is to affect the final result, the increase in isoniazid concentration after administration of PAS-isoniazid must be considerably greater than the chronologic variations.

For estimation of the free isoniazid, the chlorodinitrobenzene method has been chosen, although it implies a considerable margin of error. However, by choosing this specific reagent, one can be sure that PAS, which may be present in concentration 20 to 100 times higher than isoniazid, will not bias the results, as might occur with the use of less specific reagents despite elimination of the PAS prior to the colorimetric determination. The margin of error of the isoniazid estimation is particularly great in the presence of low concentrations, below 0.1 mg. per 100 ml. Certain discrepancies, e.g., poor consistency of the values in the plasma and in the four-hour urine, can be attributed to the fact that the estimated isoniazid concentrations are low and not entirely reliable e.g., patients *At*, *He*, *Ma* in figure 2, and *Pr*, *Ro*, *Rd* in figure 3. These doubtful values have not been discarded, so that the statistical calculation of the significance is not biased by an arbitrary choice of values.

Figures 1 to 4 indicate that some patients excrete small amounts of free isoniazid, while others excrete rather large amounts. Evidently, the first group will particularly benefit from the administration of PAS or benzoyl-PAS.

A further reason why only some patients might benefit from PAS with respect to the inactivation of isoniazid is that the possible effects of PAS as judged by studies in the rabbit are concerned only with the acetylation of isoniazid (7). In many human subjects, however, isoniazid is also split to a large extent into isonicotinic acid (1, 22). In previous, unpublished tests, the present writers have established that on the administration of PAS the breakdown of isoniazid to isonicotinic acid is not influenced. It is therefore readily seen that both PAS and benzoyl-PAS are effective only in persons in whom acetylation predominates in the processes of inactivation. Despite the considerable individual variations in the degree by which the isoniazid metabolism is affected by PAS, an effect results in tests *1*, *2*, *4*, and *6*, with a low probability of error ($P < 0.01$ to $P < 0.03$).

Kourilsky and associates (23) state that administration of the molecular isoniazid-PAS compound produces higher blood concentrations of free isoniazid than does isoniazid alone. According to investigations of Bünger and co-workers (24), as well as those of the present writers, the molecular compound is split even in an aqueous solution. In view of the small amount of PAS which thus results, it is not surprising that with isoniazid-PAS no significant increase of the excreted free isoniazid has been found. Similar findings have been reported by Gernez (25).

On the whole, the findings in the present study are consistent with those of Morse (10), Bell and associates (11), Mandel and co-workers (9), and Grosset and associates (12). These investigators made use of a microbiologic method of determination for free isoniazid, and presumably eliminated the influence of PAS by adding para-aminobenzoic acid. In fact, the method yields only gradations of concentration of 1:2:4, et cetera. After administering PAS-isoniazid, they found twice the normal isoniazid concentration in most cases and, in a few cases, four times the

normal isoniazid concentration. The effects of PAS thus observed are greater than those reported in the present study. This may be due to the great gradations of concentration characteristic of the microbiologic method.

In a number of patients, the addition of PAS does not increase the free isoniazid, a fact that has been observed in most investigations except those of Grosset and associates (12). In the various studies, the number of patients in whom no effects on free isoniazid content were produced by the addition of PAS were: 7 of 18 patients (Morse (10)); 6 of 20 patients (Bell and associates (11)); and 5 of 25 patients (Mandel and coworkers (9)). In the present studies, the addition of PAS failed to increase the free isoniazid in 8 of the 30 subjects. It is possible that, in these patients, the part played by acetylation is insignificant as compared with that of the breakdown to isonicotinic acid and that, therefore, PAS proves to be practically ineffective.

During investigations with isoniazid-PAS, Peters (14) has made use of a colorimetric determination in his tests on the excretion of free isoniazid. In four subjects, he found the same amounts of free isoniazid in the urine, regardless of whether they were given isoniazid alone or in combination with 4 gm. of PAS three times daily. Likewise, the total isonicotinic acid derivatives show no manifest connection with the administration of PAS. If, on the basis of these data, the free isoniazid is calculated in per cent of the total isonicotinic acid derivatives, slightly increased values for free isoniazid (122 to 128 per cent of the controls receiving isoniazid alone) are found in three of eight tests in which PAS was administered. In one case, a decrease to 88 per cent of the controls has occurred.

For these findings which differ so completely from those of the present study, no adequate explanation has been found, unless it be assumed that Peters might have worked with four subjects who preferentially split isoniazid to isonicotinic acid so that their isoniazid metabolism was not influenced by the addition of PAS.

Johnson (7) was the first to show the inhibition of isoniazid acetylation by means of PAS *in vitro* on incubating isoniazid and PAS with sodium acetate and liver homogenate. On substituting PAS by benzoyl-PAS, under the same experimental conditions, a similar inhibition of isoniazid acetylation has been found (unpublished tests). Since, on incubating benzoyl-PAS with liver homogenate, PAS is split off enzymically (26), it can be admitted that under these experimental conditions the released PAS induces a competitive inhibition of isoniazid acetylation. Likewise, *in vivo*, benzoyl-PAS is split to about 50 per cent (26). In view of the available tests, it cannot yet be decided, however, whether the released PAS or the benzoyl-PAS itself causes the abovementioned inhibition of isoniazid inactivation.

The *significance* of isoniazid inactivation in the treatment of tuberculosis has been discussed repeatedly. Bönicke and Orlowski (27) found no connection between the degree of the excreted free isoniazid and the therapeutic success of the medication. On the other hand, Mitchell and Bell (5) have reported that, in patients with high plasma concentrations of biologically active isoniazid, tubercle bacilli disappear from the sputum more rapidly and more frequently. In an investigation involving 100 cases of tuberculosis, Vivien and associates (6) have observed that, in patients with a serum concentration of at least 0.5 γ of free isoniazid per ml., quick recoveries occur more frequently than in patients with low serum concentrations of isoniazid.

It is a well-recognized fact that multiple-drug therapy with isoniazid and PAS constitutes one of the most effective forms of treatment of tuberculosis. The effect of PAS may be partly due to the inhibition of isoniazid inactivation. It must, however, be kept in mind that PAS also acts as an antimicrobial agent and in so doing delays the emergence of isoniazid-resistant strains of tubercle bacilli. Therefore, PAS must be administered in sufficiently high doses. The abovementioned findings on the minimal effective doses of benzoyl-PAS for inhibiting acetylation do not justify the prescription of lower dosages than those found to be effective in the clinic.

Summary

A method is described for the colorimetric determination of the ratio of free isoniazid to the total isonicotinic acid derivatives in body fluids in the presence of PAS.

It has been confirmed that isoniazid inactivation is relatively constant in the individual.

In groups of 12 to 20 patients with pulmonary tuberculosis, it was found that the addition of oral doses of either PAS or benzoyl-PAS to isoniazid therapy induces a significant increase in the concentration of free isoniazid in the plasma and urine in the majority of cases. The average

of the values of all of the cases also demonstrated an increase.

The minimal effective dose of calcium benzoyl-PAS for significantly augmenting the excretion of free isoniazid was found to be 8 gm. per day. Smaller doses of benzoyl-PAS, as well as the small PAS fraction of the molecular isoniazid-PAS compound, are ineffective.

Sumario

La Inhibición de la Inactivación de la Isoniacida por Medio del PAS y del PAS Benzoílico en el Hombre

Describese un método para la determinación colorimétrica de la proporción de isoniacida libre en relación con el total de derivados del ácido isonicotínico en los humores orgánicos, en presencia de PAS.

Se ha confirmado que la inactivación de la isoniacida es relativamente constante en el individuo.

En grupos de 12 a 20 tuberculosos pulmonares, se observó que la adición de dosis orales ya de PAS o de PAS benzoílico a la isoniacidoterapia provoca en la mayoría de los casos un aumento importante de la concentración de isoniacida libre en el plasma y la orina. El promedio de los valores de todos los casos también reveló aumento.

La dosis eficaz mínima de PAS-benzoílico cálcico para aumentar significativamente la excreción de isoniacida libre resultó ser de 8 gm. al día. Dosis más bajas de PAS benzoílico, así como la pequeña fracción de PAS en el compuesto molecular de isoniacida-PAS, resultan ineficaces.

Resume

L'inhibition de l'inactivation de l'isoniazide au moyen du PAS et du benzoyl-PAS chez l'homme

Description d'une méthode pour la détermination colorimétrique du rapport entre l'isoniazide libre et la totalité des dérivés de l'acide isonicotinique dans les liquides organiques en présence de PAS.

Il a été confirmé que l'inactivation de l'isoniazide est relativement constante chez l'individu.

Dans des groupes de 12 à 20 patients atteints de tuberculose pulmonaire, il a été observé que l'addition de doses orales de PAS ou de benzoyl-PAS à l'isoniazide entraînait dans la majorité des cas une augmentation significative de la concentration de l'isoniazide libre dans le plasma et l'urine. La moyenne des valeurs relevées dans tous les cas démontrait également cette augmentation.

La dose minima efficace de benzoyl-PAS calcique qui s'est montrée nécessaire pour produire l'augmentation notable de l'excrétion d'isoniazide libre est de 8 g par jour. Les doses plus faibles de benzoyl-PAS, de même que la petite quantité de PAS du composé moléculaire isoniazide-PAS sont inefficaces.

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MECHANICS OF BREATHING[1]

I. The Physical Properties of the Lung

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(Received for publication September 12, 1958)

Introduction

The primary function of the lung consists in providing the organism with an adequate oxygen supply and carbon dioxide elimination. This involves a number of intricate steps. The first of these comprises the gas exchange between environment and interior of the lung, the ventilatory function. The second stage involves the gas exchange across the alveolo-capillary membrane, commonly referred to as the diffusion capacity of the lung. Finally, the diffusing gases have to be transported to the site of utilization (internal respiration) in the case of oxygen and to the site of elimination (lungs and kidneys) in the case of carbon dioxide. Thus, an efficient gas exchange in the lungs is only possible if the three different functions, namely, ventilation, diffusion, and circulation, are intact. This and the two subsequent papers deal essentially with the ventilatory stage and its mechanical effects upon gas exchange and circulation.

The ventilatory mechanism follows the physical laws of a pump. A pressure gradient is necessary to provide gas flow between the atmosphere and the interior of the lung. During inspiration, the pressure within the lung is negative with regard to the atmosphere, and air is drawn into the lungs. The negative pressure is created by the effort of the inspiratory muscles and the elasticity of the chest wall during expansion of the lungs. In expiration, the pressure within the lungs is positive with regard to the atmosphere. In normal breathing, this is a passive process, the necessary pressure for expiratory airflow being provided by the elastic energy stored in the lungs during inspiration. However, if the elastic forces of the lungs become too small to provide the pressure gradient required for expiration, e.g., in chronic bronchial asthma, chronic pulmonary emphysema, exercise, et cetera, the expiratory muscles have to carry a large part of the expiratory work load. The pressure gradient necessary for a given tidal volume depends upon the physical properties of the lungs (1–6). These will be discussed as the elastic properties, the resistive properties, and the over-all time factor.

If the lungs are considered as a pair of bellows connected with a handle (functioning analogous to the intrapleural space) and fixed by an elastic spring (7), three different forces acting between the lungs and the intrapleural space can be distinguished. These forces are balanced by the sum of the thoracic forces: the muscular forces of the chest wall, the elasticity of the chest wall, and the viscosity of the chest wall. The spring represents the elasticity of the lung tissue, the fabric depicts the viscosity of the lung tissue, and the openings in the bellows portray the airways. If the handle is pulled, the bellows are distended and air enters as long as the distention is continued. If the function of the thoracic cage is impaired, i.e., weakness of respiratory muscles, the volume changes are reduced even though the physical properties of the lungs are normal. As a result, vital capacity and maximal breathing capacity will be reduced.

During the distension of the bellows, the elastic spring and the resistance of the bellows system, namely resistance to deformation (viscosity) and to airflow, have to be overcome. Once the bellows are distended and the handle is released (the inspiratory muscles relax), the bellows will collapse, provided the elastic recoil of the spring is sufficient to overcome the mechanical resistance of the system (passive expiration). If the spring is too weak, the handle has to be pushed in order to overcome tissue viscosity and airflow resistance. It is apparent that either a decrease in elastic recoil or an increase in mechanical resistance can lead to the same result, namely, an active expiration.

The Elastic Properties

The elastic properties determine the static pressure necessary for a given volume change. A

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normal lung requires approximately a pressure of 5 cm. of water for a volume change of one liter. The elastic properties of the lung are commonly expressed as compliance, i.e., volume change per unit pressure change (L/cm.H_2O) or its inverse elastance: pressure change per unit volume change (cm.H_2O/L). For a pressure gradient of 5 cm. of water per liter change in lung volume, the compliance amounts to 0.200 L/cm.H_2O and the elastance to 5 cm.H_2O/L. A low compliance, therefore, characterizes a stiff lung (difficult to distend); and a high compliance, an easily distensible lung. A good example of a stiff lung (low compliance) is represented by a congested or fibrotic lung, while an emphysematous lung is characterized by a high compliance. In practice, compliance is determined by dividing the volume change, usually the tidal volume, by the static intrapleural or intra-esophageal pressure difference between points of zero airflow (end-expiration and end-inspiration).

However, it must be realized that the elastic properties of a three-dimensional structure such as the lung are more correctly expressed by the modulus of elasticity (5). The modulus of elasticity for the lung is given by the following equation:

$$\text{Mod } E_L = \frac{\Delta p_{el}}{\frac{\Delta V_L}{V_L}} = \frac{V_L}{C}$$

where

Δp_{el} = elastic pressure difference for a given volume change (ΔV_L)
V_L = initial lung volume
C = compliance

It follows that the greater the lung volume, the higher the compliance. This fact explains that compliance varies with the physical characteristics of the individual studied. As a corollary, there is a close relationship between vital capacity and compliance (8, 9).

The elastic properties of the lungs are the result of a number of various factors. Basically, these properties are due to the elastic fibers in the alveolar walls. The inspiratory stretching of these fibers provides the elastic energy necessary for a normal expiration. Because the elastic force of the alveolar wall is a function of its distention, it is obvious that the total elastic force of the lung (lung tension) depends upon the number of ventilated alveoli. A small lung consists of less alveoli than a larger lung and requires, therefore, a greater pressure gradient for a given volume change. Thus, the slope of the pressure-volume curve of the lungs varies with the lung volume. Below the functional residual capacity, a greater pressure is required for a given volume change than in the subject's usual breathing range. Greater pressures are also necessary toward the maximal inspiratory position, where the alveolar walls reach the limit of their distensibility.[3]

With increasing age, the static volume-pressure curve of the lungs shifts toward the left, indicating a decreased elastic recoil. This is probably associated with an alteration of the elastic properties of the thorax, leading to changes in the lung volume and the mechanical behavior of the lungs similar to those in emphysema, though on a much smaller scale (10).

If the air volume of the lungs is diminished as in edema, pleural effusion, tumors, pneumonia, or atelectasis, the compliance decreases not primarily because of a change in the properties of the elastic fibers, but because the number of functioning alveoli is decreased.

Once an alveolus or a group of alveoli become airless, much larger pressures are necessary to reinflate them. This is due to a change in surface tension forces between the air-liquid phase. It has been demonstrated that the pressures necessary to reinflate "closed off" alveoli with saline are considerably less than the pressure required for reinflation by air (11). This explains the difficulties encountered in trying to inflate atelectatic areas by purely mechanical means alone and provides at least some theoretic support in favor of the use of wetting agents in such conditions. The role of capillary erection for the inflation of the lung of newborns has recently been stressed (12). Surface forces have also been invoked for the explanation of hysteresis (11) during the establishment of pressure-volume curves and for uneven filling of the lungs. Changes in surface forces and a decrease in lung volume available for air account for changes in the elastic properties of the congested lung (13–15). Above the functional residual capacity, an edematous lung becomes stiffer and, below the functional residual capacity, more distensible than a normal lung (16). These factors also are

[3] These relationships are expressed in Laplace's law, which states that the wall tension for a hollow sphere is proportional to the internal pressure and the radius of the sphere.

responsible for some of the changes observed during changes in body position (17, 18). Finally, the measured elastic properties of the lung are influenced by the amount of unequal ventilation. The latter may be caused by disturbances in the chest wall, lung parenchyma, or the bronchial pathways.

In any case, the normal lung segments are overventilated in comparison with the diseased parts. This disproportion in air distribution is accentuated further during rapid breathing. Because there is no time for equilibration of pressures during the periods in which no airflow exists (and elastic pressures are measured), the compliance decreases, reflecting the overextension of the well-ventilated alveoli. Thus, this again represents a stage in which the functioning lung volume is diminished. However, if enough time is allowed for equilibration of pressures at the end of inspiration and expiration, the effects of uneven ventilation on compliance are abolished and the *true static compliance* can be determined. In a dyspneic patient, this may be technically impossible because of his inability to hold a breath until complete equilibration of pressures has occurred.

Some confusion has arisen because of this concept which centers around the terms of static and functional compliance (19, 20). *Static compliance* expresses the true compliance of the lungs as a whole and is determined as the pressure change per unit volume change between points of zero airflow (usually end of inspiration and end of expiration) after equilibration of intrathoracic pressures. In the normal lung, this pressure equilibration is instantaneous, and compliance measurements in this condition represent the true compliance of the lungs. In the presence of unequal ventilation, however, intrathoracic pressures are not yet equilibrated at the points of zero airflow, and the pressure difference between end-expiration and end-inspiration represents primarily the compliance of the well-ventilated alveoli plus a flow-resistive element. This has been termed *functional compliance*. It is easily recognized if the compliance is measured at two different respiratory rates.

The significance of functional compliance has been illustrated in a patient with chronic bronchial asthma (18). During normal respiration, his functional compliance was 0.1 L/cm.H_2O. However, after the respiratory rate had been reduced by introducing an inspiratory pause, a static compliance of 0.17 L/cm.H_2O could be determined. This increase in compliance is associated with better distribution of the inspired air (18).

The presence of unequal ventilation is one of the major functional disturbances in chronic pulmonary emphysema (21–23). In this condition, it may be impossible to determine the static compliance. Usually, the value for functional compliance is low and there might be an impression of dealing with a physiologically stiff lung. This is, however, not the case. Due to alveolar dehiscence, the emphysematous lung is actually more distensible than a normal lung, and its static compliance is higher than normal. This diminished elastic recoil is quite impressive at autopsy. Despite the open thorax, the emphysematous lung collapses very little. The low functional compliance reflects primarily the physical properties of the well-ventilated areas and not of the *emphysematous lung* as a whole.

The decrease in elastic recoil in the patient with emphysema becomes extremely unfavorable during expiration. In the normal lung, the elastic forces of the alveoli tend to keep the airways patent against the positive intrathoracic pressure during the expiratory phase. If these elastic forces are decreased, their pull becomes insufficient and the airways collapse during expiration (ball valving). This initiates automatically a greater expiratory effort which, in turn, promotes more ball valving. Thus, a vicious cycle is established in which precious energy is wasted in futile efforts.

The Resistive Properties

Resistive properties determine the dynamic pressures necessary to move air in and out of the lung. Essentially, these resistive forces have to overcome the resistance of the airways and the resistance to tissue deformation. In contrast to the elastic forces, they are time dependent and a function of the speed at which the volume change occurs. They are usually measured together and expressed as mechanical resistance, i.e., the pressure necessary to produce a given airflow (cm.H_2O/L/sec.). The resistance to tissue viscosity has been estimated between 10 and 20 per cent of the mechanical resistance (24, 25). In diseases involving the lung parenchyma, it is often raised, but the increase is seldom a major part of the respiratory work (26). Airflow resistance is usually the determining factor for the total mechanical resistance. A third flow resistive

force is necessary to overcome the inertia of the lung. The latter is negligible, however, up to respiratory rates of 100 per minute (27).

The mechanical resistance is usually determined by dividing the flow resistive pressure (dynamic intrapleural pressure minus static intrapleural pressure) by the airflow rate (6). Because the relationship between flow and flow-resistive pressures is not linear, mechanical resistance varies over the respiratory cycle, particularly at high flow rates. It is usually expressed as either resistance for a given flow rate or as mean mechanical resistance for the whole respiratory cycle. In the latter case, the individual resistance values are integrated with respect to time.

Based upon the nonlinear pressure-flow relationship, Clements (28) has developed a very ingenious device for measuring mechanical resistance by means of rapidly interrupting airflow (6). Putting an external resistance of known value with the same pressure-flow characteristics as the lung in series with the airways, mechanical resistance can be directly read off from a pressure gauge. The method is based upon the assumption that the flow-resistive properties of the chest wall are negligible with respect to the flow-resistive properties of the lung. If the initial good results of this method can be confirmed in large comparisons, this may become a choice office procedure.

A very elegant method of measuring airway resistance alone has been described by Dubois (29, 30). Based upon Boyle's law, the intrathoracic gas volume is measured by measuring volume changes in a body plethysmograph and plotting them against changes in mouth pressures during inflation and/or deflation of the chest while the airways are completely obstructed at the mouth. In contrast to the determination o

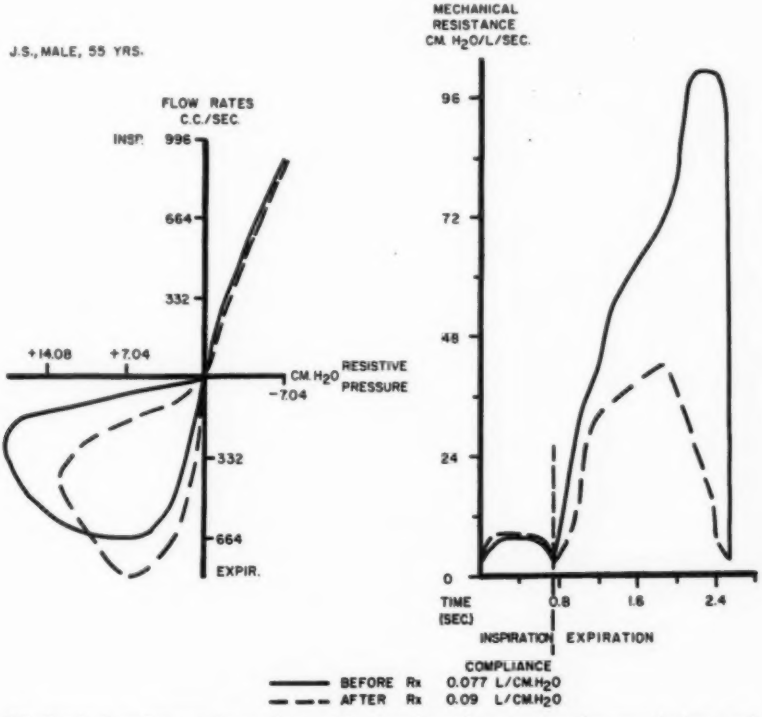

FIG. 1. Mechanical resistance in patient with chronic pulmonary emphysema before and after aminophyllin, 0.25 gm. intravenously.

TABLE 1

THE MECHANICS OF BREATHING IN 25 NORMAL SUBJECTS AND 75 PATIENTS WITH CARDIOPULMONARY DISEASE (SUPINE POSITION)

(Mean and Standard Deviation)

	Number of Subjects	Compliance SR* L/cm. H_2O	Compliance FR† L/cm. H_2O	Mean Inspiratory Resistance cm. H_2O/L/sec.	Mean Expiratory Resistance cm. H_2O/L/sec.
Normal subjects	25	0.162 ± 0.04	0.150 ± 0.047	2.95 ± 1.34	3.45 ± 1.51
Chronic bronchial asthma	10	0.106 ± 0.041	0.081 ± 0.038	11.9 ± 10.8	16.86 ± 14.8
Chronic pulmonary emphysema	26	0.130 ± 0.082	0.058 ± 0.03	9.73 ± 6.44	17.54 ± 11.42
Restrictive pulmonary disease	10	0.083 ± 0.041	0.075 ± 0.041	5.24 ± 3.45	7.5 ± 3.72
Pulmonary fibrosis	5	0.072 ± 0.042	0.065 ± 0.051	5.56 ± 4.66	10.84 ± 9.38
Allergic bronchitis	2	0.133	0.078	2.9	4.95
Cardiovascular disease	22	0.090 ± 0.043	0.070 ± 0.032	4.91 ± 2.45	7.45 ± 4.46

* Slow respiratory rate.

† Fast respiratory rate.

functional residual capacity by methods based upon intrapulmonary mixing, the plethysmographic method measures the total intrathoracic gas volume, including the nonventilated areas. It has been found that the functional residual capacity in patients with chronic pulmonary emphysema may thus be considerably larger than the one determined by one of the conventional dilution methods (31). The difference of the value obtained by the two methods represents nonventilated intrathoracic gas volumes, such as closed cysts, pneumothorax, et cetera. Once the relationship between alveolar pressure change and plethysmograph pressure change has been determined (during measurement of intrathoracic gas volume), airflow resistance is readily measured by plotting airflow rates against mouth pressures and using appropriate corrections for the dimensions of the measuring system.

Because the caliber of the airways is a function of lung volume, mechanical resistance is considerably higher at low lung volumes than at high lung volumes. In the normal breathing range, inspiratory resistance and expiratory resistance are about equal in normal lungs. However, at low lung volumes, the expiratory resistance increases considerably more than the inspiratory resistance. Normally, 54 per cent of the total resistance is located in the upper airways from the nose and mouth to the tracheal bifurcation, and only 46 per cent in the bronchial airways (3).

The marked increase in flow resistance, which is seen in bronchospastic disease, is due to a change in the characteristics of the lower airways. Under these conditions, expiratory flow resistance is considerably more increased than inspiratory flow resistance. This discrepancy is in part due to ball valving. The large increase in mechanical resistance over the whole respiratory cycle in patients with bronchospastic disease may be seen in figure 1 and table 1. While the normal subject shows no change in flow resistance from inspiration to expiration, the patients with chronic bronchial asthma or chronic pulmonary emphysema demonstrate a marked increase in inspiratory resistance and an even more impressive increase in expiratory resistance. The latter rises continuously during the expiratory cycle in spite of decreasing flow rates, thereby forming a loop instead of a straight line. As expiration proceeds, higher and higher pressures are required to move the air out of the lungs at a minimal flow rate. This is a typical example of the unfavorable effects of ball valving. In this particular instance, there was little relief of bronchial obstruction by 0.25 gm. of aminophyllin intravenously.

THE OVER-ALL TIME CONSTANT OF THE LUNGS

The lungs can be considered as a series of composite elements, each consisting of an alveolar sac and a bronchial tube. The mechanics of the air sacs are primarily governed by its elastic properties while the mechanics of the bronchial airways are determined by its flow-resistive properties. The common characteristic for such a

system is its time constant, i.e., the product of compliance and mechanical resistance, which has the dimensions of time. If the time constants of the individual pathways are unequal, the distribution of ventilation becomes a function of respiratory rate. The higher the respiratory rate, the more the ventilated air follows the pathways of least resistance, i.e., the low-resistance highly compliant areas (a more normal segment of lung). Thus, the normal lung segments become overventilated while the others are underventilated. In addition, part of the air will travel back and forth between well-ventilated and poorly ventilated areas (Pendelluft), thus serving no useful purpose for pulmonary gas exchange. This mechanism leads to the well-known difficulties encountered in the pulmonary gas exchange in the presence of uneven ventilation, when the overventilated areas are unable to compensate for the disturbances of the poorly aerated sections.

The results in 25 normal subjects and in 75 patients with cardiopulmonary disease studied in the supine position (9) are summarized in table 1. It may be seen that compliance and mechanical resistance of the normal subjects is independent of respiratory rate, the respective values being 0.162 plus-minus 0.040 L/cm. H_2O and 2.8 plus-minus 1.3 cm. H_2O/L/sec. In contrast, the patients with chronic bronchitis, bronchial asthma, and pulmonary emphysema show a marked decrease of compliance as the respiratory rate is increased, thus indicating markedly unequal ventilation. These diseases are also characterized by an increase in mechanical resistance, the increase being considerably greater during expiration than during inspiration. Restrictive pulmonary disease and pulmonary fibrosis, on the other hand, show a decrease in compliance, which is relatively independent of respiratory rate, and a somewhat increased mechanical resistance. The patients with heart disease demonstrate similar characteristics.

From these data the relationships between vital capacity and maximal breathing capacity, on the one hand, and the physical characteristics of the lungs, on the other hand, have been plotted (9). With the exception of chronic pulmonary emphysema, there is a direct relationship between vital capacity and compliance. Because of the abnormal conditions of the alveolar structure in chronic pulmonary emphysema, a discordance between vital capacity and compliance would be expected in this disease. For all of the other groups, the vital capacity represents a rough estimate of the pulmonary compliance, the compliance per liter in vital capacity ranging from 0.03 to 0.039 L/cm. H_2O/sec. With decreasing vital capacity, the mechanical resistance tends to increase. This is primarily an expression of the decreased caliber of the airways associated with small lung volumes.

Mechanical resistance or the over-all time constant correlates poorly with either the maximal breathing capacity or the air velocity index. This is understandable if it is realized that the conventional tests of ventilation (maximal breathing capacity, timed vital capacity, air velocity index) measure the over-all function of the lung-thorax system. As mentioned earlier, a muscular weakness or deformation of the chest wall may decrease the driving force of the ventilatory bellows without necessarily changing the physical characteristics of the bellows themselves. Hence, a reduction in maximal breathing capacity or the air velocity index does not necessarily imply an increased resistance to airflow, but may well be due to either a central or local reduction of the driving force.

Summary

The physical properties of the lung are divided into an elastic and a flow-resistive component. These two properties are related by the over-all time factor of the ventilatory system.

Pulmonary compliance and airflow resistance are analyzed with the help of a simple model, and their changes in cardiopulmonary disease are evaluated. The dependence of these properties upon lung volume and airflow rate is stressed, and the relationship of these factors to the commonly used ventilatory function tests are discussed.

Sumario

La Mecánica de la Respiración. I. Las Propiedades Físicas del Pulmón

Las propiedades físicas del pulmón se dividen entre un componente elástico y otro resistente a la corriente de aire. Esas dos propiedades se hallan relacionadas por el factor cronológico global del sistema ventilador.

Con la ayuda de un modelo sencillo se analizan la colaboración pulmonar y la resistencia a la corriente de aire, justipreciándose sus alteraciones en las enfermedades cardiopulmonares. Recálcase

la dependencia en que se encuentran esas propiedades con respecto al volumen pulmonar y a la velocidad de la corriente de aire y se discute la relación que guardan estos factores con las pruebas de la función pulmonar, usadas habitualmente.

RESUME

La mécanique respiratoire. I. Les propriétés physiques du poumon

Les propriétés physiques du poumon se répartissent en deux composants, l'élasticité et la résistance à l'écoulement de l'air. Ces deux propriétés sont liées par le facteur temps du système de ventilation.

L'élasticité pulmonaire et la résistance à l'écoulement de l'air sont analysés à l'aide d'un modèle simple et leur variation dans la maladie cardio-pulmonaire sont évaluées. La dépendance de ces propriétes sur le volume pulmonaire et le taux d'écoulement de l'air est mise en évidence et la corrélation entre ces facteurs et les tests classiques de la fonction ventilatoire sont étudiés.

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MECHANICS OF BREATHING[1]

II. The Respiratory Work

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(Received for publication September 12, 1958)

Introduction

In the previous paper (1), the physical properties of the lungs and their relation to lung volume, surface tension forces, and unequal ventilation were discussed. Although the study of pulmonary compliance and mechanical resistance has contributed tremendously to the understanding of ventilatory function, these factors represent only single parameters of a function and by no means the function itself.

To determine the efficiency of ventilation, the respiratory work must be measured in relation to oxygen uptake in the lungs.[3] By this method, an objective appraisal of cost and result can be obtained which is independent of the cooperation of the subject studied. This is not the case if ventilation alone is measured in relation to oxygen uptake (conventional studies).

Observations

The interplay of the respiratory forces can be expressed by the following formula (1):

$$P_{ipl} = P_{l_{el}} + P_{l_{re}} + P_{ace}$$
$$= P_m + P_{th_{el}} + P_{th_{vi}}$$

P_{ipl} = intrapleural pressure
$P_{l_{el}}$ = elastic pressure of the lung
$P_{l_{re}}$ = flow resistive pressure of the lung
P_{ace} = acceleration pressure of the lung
P_m = muscle pressure of the thoracic cage
$P_{th_{el}}$ = elastic pressure of the thoracic cage
$P_{th_{vi}}$ = viscous pressure of the thoracic cage

It has already been pointed out that the acceleration pressures (necessary to overcome the inertia of the lung) are negligible at respiratory rates up to 100 per minute. Thus, the intrapleural pressure has essentially two components with respect to pulmonary pressures:

1. The static component, *elastic pressure*, is a function of lung volume. This pressure is measured at points of zero flow and represents pulmonary compliance in relation to tidal volume.
2. The dynamic component, *flow resistive pressure*, is a function of airflow rate.

On the other hand, the intrapleural pressure is equal to the sum of pressures acting on the thoracic cage. This includes the diaphragm and the abdominal muscles and contents. The direction and the size of the individual forces depend upon the respiratory phase, the distention of the lung, and the speed at which the lung volume changes.

The inspiratory work is done primarily by the respiratory muscles. Part of it, however, is performed passively by the elastic energy stored in the thoracic cage. The latter decreases with increasing lung volume. During normal breathing, the elastic energy of the inflated lungs is adequate to take care of the resistive expiratory work. Active expiratory work is required only if the latter is increased out of proportion to the available energy. The increase in expiratory flow resistance during moderate hyperventilation or airway obstruction results in a higher respiratory midposition. Thus, the potential elastic energy increases, allowing a passive expiration even under these conditions. Only if the elevated flow resistance cannot be met by the potential elastic energy, i.e., loss of elasticity in chronic pulmonary emphysema, maximal hyperventilation or airway resistance, the expiratory respiratory musculature becomes active. The maximal work available for breathing is defined by the pressure-volume diagram of the chest and amounts roughly to 10 meter-kilograms (2).

Work equals pressure times volume. During quiet breathing, the total respiratory work is approximately 0.5 mkg. per minute (3). By plotting transpulmonary pressure (intrapleural pressure minus mouth pressure) against volume,

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[3] During diffusion respiration, the respiratory work is zero, despite an adequate oxygen uptake. The fallacies of this concept will be dealt with in the third paper of this series (19).

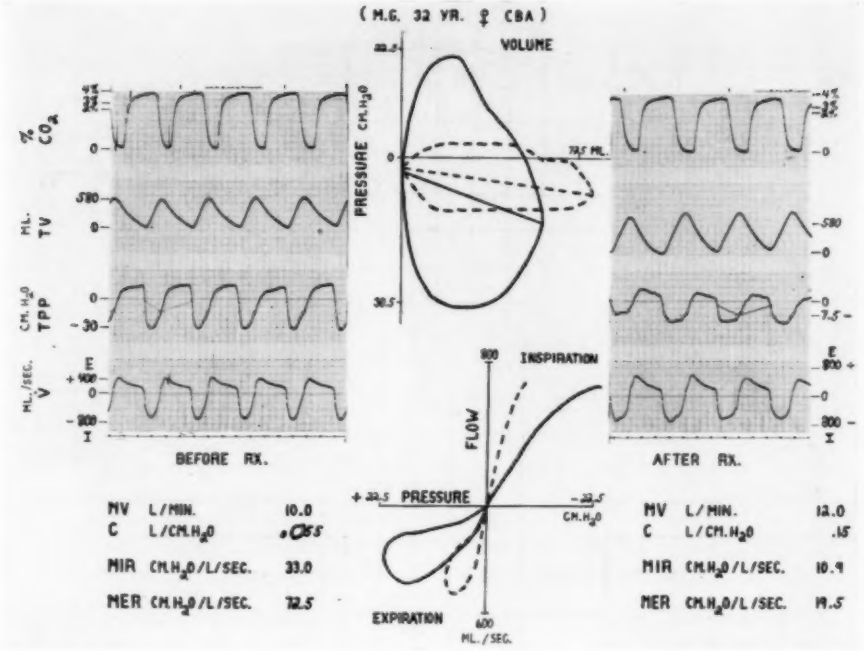

FIG. 1. Influence of bronchodilator aerosol (Dylephrin® 6 isoniazid) upon ventilatory mechanics.

a loop is obtained which represents the respiratory work done on the lungs. Transpulmonary pressures are plotted against the corresponding volumes at intervals of one-tenth of a second and the loops (top center of figure 1) are obtained. In these loops, the elastic work is expressed by the rectangular triangle, whose hypotenuse runs from the end-expiratory to the end-inspiratory pressure (diagonals). The resistive inspiratory work is represented by that part of the loop which extends from the hypotenuse downward. The active resistive expiratory work is equal to the part of the loop extending from a horizontal drawn through the points of end-expiratory pressure (the beginning of the diagonals in figure 1) upwards. While the expiratory work is less than the elastic work for the normal subject, it is considerably greater in patients with chronic bronchial asthma and chronic pulmonary emphysema.

The decrease of respiratory work in a patient with chronic bronchial asthma after the inhalation of an effective bronchodilator aerosol is also demonstrated in figure 1. In spite of an increase in tidal volume and airflow rates, the transpulmonary pressure gradient is considerably lower. As a result, compliance rises while mechanical resistance and respiratory work decrease.

The improvement of ventilatory mechanics after a bronchodilator aerosol during a vital capacity maneuver in the same patient is shown in figure 2. An increase of the vital capacity by 50 per cent is achieved although the transpulmonary pressure gradient remains essentially unaltered.

In figure 1 it may be seen that the treatment was followed by a marked reduction in airflow resistance. This was not associated, however, with an increase in the ratio first second/total vital capacity. These two figures also demonstrate a small but appreciable reduction in end tidal carbon dioxide (top tracing).

Normally, the flow resistive work amounts to only 38 per cent of the total work (1). It is demonstrated in figures 1 and 3 that this proportion is considerably increased during bronchocon-

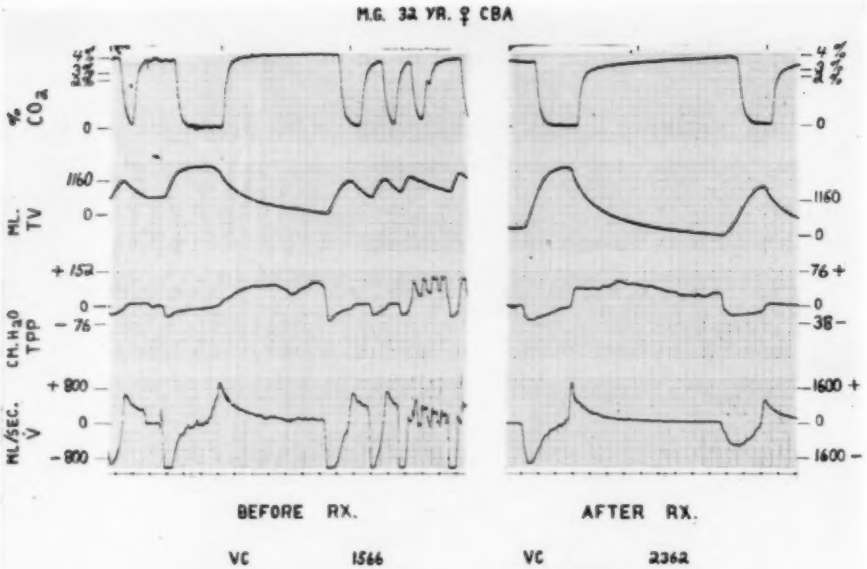

FIG. 2. Influence of bronchodilator aerosol (Dylephrin® 6 isoniazid) upon ventilatory mechanics during vital capacity maneuver.

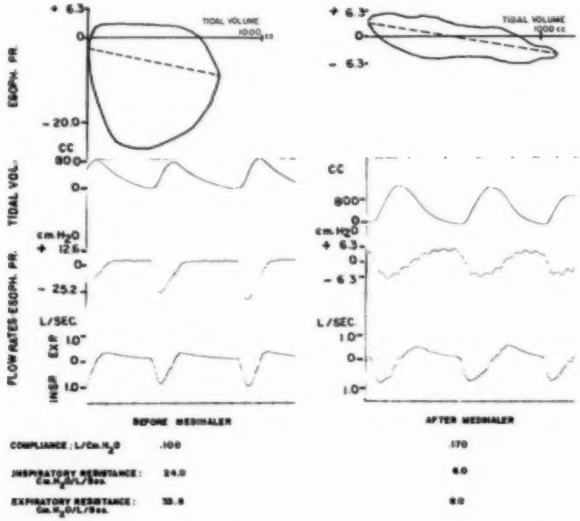

FIG. 3. Mechanics of breathing in a patient with chronic bronchial asthma (H.P., 44-year-old-male) before and after medihaler isoproterenol inhalations ×6.

striction. *In patients with pulmonary disease, the respiratory work may be increased to such an extent that most of the energy expenditure goes into respiration alone, thereby depriving the organism of any energy necessary for even slight physical work.*

It has been shown that the ventilatory response to carbon dioxide inhalation can be limited by the physical properties of the lung-thorax system (5). On the other hand, the gas exchange can be improved even in the presence of reduced ventilation if part of the respiratory work is taken over by a self-cycling respirator (6). The latter decreases the total metabolism by reducing the work of the respiratory muscles. As a result, carbon dioxide output becomes smaller and the alveolar carbon dioxide pressure (pCO_2) can be lowered despite a reduction in ventilation.

All of the methods described for measuring pulmonary respiratory work completely neglect the metabolic work necessary to keep the lung inflated at a given functional residual capacity. This may constitute a major part of the respiratory work in certain cases, particularly at high lung volumes. This part, however, is difficult to evaluate: first, because part of the energy required to keep the lung distended at its residual volume stems from the elastic energy of the rib cage; and, second, because intrapleural pressure measurements are usually substituted by intraesophageal pressure measurements. Although the latter have been proved to reflect intrapleural pressure variations faithfully under proper experimental conditions (7–11), the absolute pressures are always different by an unpredictable amount. The latter depends upon position and inflation of the balloon and the characteristics of the mediastinum (12).

McIlroy (13) has described two simplified methods of measuring pulmonary respiratory work. The first of these methods is based upon the constancy of the phase relationship between pressure and volume change. It measures only inspiratory work by the formula:

$$W = k\Delta p\dot{V}_E$$

where:

- W = respiratory work during inspiration.
- Δp = difference between end-expiratory pressure and maximal inspiratory pressure.
- $\dot{V}_E$ = minute ventilation.
- k = constant taken as 0.7 if the ratio between elastic pressure difference and maximal inspiratory transpulmonary pressure difference is more than 0.75; and as 0.8 if the ratio is less than 0.75. Although this method may be quite reliable in humans, it certainly overestimates the respiratory work in dogs, whose lungs have extremely low flow-resistive properties (14).

The second and most accurate method of McIlroy is based on the assumption that intraesophageal pressure and tidal volume are similar functions of time. This method is comparable with the one used in calculating cardiac work. The inspiratory work is derived by the product of mean inspiratory intrapleural pressure and minute ventilation $\frac{(\int Pdt \times MV)}{t}$, and the expiratory by the product of mean expiratory intrapleural pressure minus end-expiratory intrapleural pressure and minute ventilation

$$\frac{((\int Pdt - \text{end exp. p})\ MV).}{t}.$$

In the experience of the present writers, this latter method has proved much more reliable in humans as well as in dogs.

For a given total ventilation, the higher the respiratory rate, the lower the mechanical work. This is so because elastic work is inversely proportional to frequency and nonelastic work independent of frequency per se.

For normal breathing when expiration is passive, the relationship between the work of breathing and ventilation may be expressed by an equation of the type proposed by Otis (3):

$$W = a\dot{V}^2/f + b\dot{V}^2 + c\dot{V}^2$$

where:

- W = respiratory work
- $\dot{V}$ = rate of ventilation
- f = respiratory rate
- a = elastance
- b, c = coefficients of nonelastic resistance

If expiration becomes active, the above equation has to be modified as follows:

$$W = 2(b\dot{V}^2 + c\dot{V}^2)$$

From these equations, it is quite obvious that the work of breathing is proportional to ventilation

only so long as the various constants (a, b, c) remain unchanged. The fallacies of interpreting respiratory work or efficiency from minute ventilation alone have been pointed out (15).

The situation is quite different if alveolar ventilation is considered. There is an optimal frequency for a given alveolar ventilation at which the work of breathing is minimal (2, 16). For the normal subject, the optimal respiratory frequency (minimal work) is about fifteen per minute. For patients with decreased compliance, the minimal work occurs at higher frequencies, while patients with increased mechanical resistance breathe at lower rates. Both at rest and during exercise, the body seems to choose frequencies close to those requiring minimal work even at the cost of alveolar hypoventilation.

The total respiratory work varies greatly at different levels (17). The normal mid-position requires the least amount of work. Below this level, the work increases mainly because of an increase in the resistive component. Above the mid-position, the work increases because of an increase in elastic work. In normal subjects, the expiratory work is greater than the inspiratory work at respiratory levels below the functional residual capacity. For patients with pulmonary disease, this discrepancy between expiratory and inspiratory work is found frequently to be independent of midposition, although it is accentuated at low respiratory levels. Ventilation can be greatly improved and the respiratory work decreased by means of properly employed pressure breathing. There are essentially two different methods of applying this form of therapy. In the first method, either the whole patient or his chest is subjected to negative pressure either by a tank or a chest respirator. The chest wall expands and air flows into the lungs. Termination of the negative-pressure phase initiates passive expiration. In the second method, positive pressure is applied to the oropharynx, thus inflating the lung-thorax system. Again, the termination of the positive-pressure phase (at the end of inspiration) starts the passive expiration.

At first glance, the first method seems to simulate the normal physiologic conditions more closely. Hoping to increase the efficiency of both methods and to decrease their side effects, an opposite pressure phase is sometimes added during expiration. A positive pressure (booster) during expiration with the body respirators and a negative pressure during expiration with the oropharyngeal pressure-breathing units supply alternating positive-negative pressures during inspiration and expiration, respectively.

In order to ventilate a subject properly, the driving force (transthoracic pressure) has to be adjusted with respect to the sum of the resistive forces in the lung-thorax system. The required amount of ventilation can be estimated from a nomogram (18); and, by measuring the actual ventilation, the driving pressure has to be properly adjusted.

Prolonged artificial ventilation is almost invariably associated with accumulation of secretions and frequently with partial atelectasis. Thus, the mechanical behavior of the lungs deteriorates during the course of prolonged treatment. As a rule, the physical properties of the thorax change considerably less. Therefore, the increase in intrathoracic pressure associated with a given volume change is essentially due to an increase in transpulmonary pressure rather than to an increase of the pressure gradient across the thoracic wall. From this fact stems an essential difference between the described two methods of artificial ventilation. If the physical properties of the lungs become worse, the intrapleural pressure variations increase markedly in the tank respirator, while they remain relatively small in the case of pressure breathing applied to the oropharynx. In both cases, the total pressure applied for a given change in lung volume obviously must be equal. Because of the marked influence of intrapleural pressure changes upon the circulation (*see* paper III (19) in this series), the circulatory effects of a tank respirator must be greater than those of oropharyngeal pressure breathing for a given pressure setting. In table 1, the relationship between the various pressure gradients and the

TABLE 1

PRESSURE COMPONENTS DURING THE TWO TYPES OF ARTIFICIAL RESPIRATION

	Tank Respirator	Oropharyngeal Pressure Breathing
	Pressure Gradient Due to Resistive Properties of:	
Total transthoracic pressure gradient	Chest wall and lung	Chest wall and lung
Intrapleural pressure	Lungs	Chest wall
Transthoracic intrapleural pressure	Chest wall	Lungs

mechanical properties of the lung-thorax system are summarized.

Otis and Riley (3, 20) have stressed the correlation between the respiratory work and respiratory acidosis. In patients with obstructive pulmonary disease, the oxygen requirements of the respiratory muscles rise so sharply that the limits of maximal ventilation are very low. Because of the high metabolic costs of breathing, the carbon dioxide production is proportionately higher than the increase in alveolar ventilation. Therefore, in a pulmonary patient, in whom the high carbon dioxide is caused by the increased work of breathing, nothing is to be gained by increasing his ventilation. The increase in ventilation would lead to a further elevation of the respiratory work and a possible rise in alveolar pCO_2. The use of respiratory stimulating drugs under such conditions would appear to be not only useless but actually harmful. The therapeutic aim should be directed against an improvement in gas exchange by relieving the respiratory work with appropriate agents, such as bronchodilators, antimicrobials, and artificial respiration (*see* figure 1).

This leads to the approach of evaluating the work of breathing by measuring its metabolic costs (21, 22). From such experiments, the cost of resting ventilation has been estimated at about 0.5 ml. of oxygen per liter ventilation. During hyperventilation, the oxygen consumption rises in the form of a parabola (22). A ventilation of 116 L/min. requires about 800 ml. of oxygen per minute. From these measurements, the efficiency of the respiratory muscles can be calculated to be 3 to 7 per cent, i.e., the muscles require between fourteen and thirty-three times more oxygen in order to perform their work than would be necessary to obtain the same amount of caloric energy (3). Because of the parabolic rise of oxygen consumption during hyperventilation, the oxygen available for nonrespiratory work decreases with increasing minute ventilation. The level at which no oxygen is available for extra work has been estimated at 140 L/min., a limit which is probably never reached during muscular exercise in normal subjects. However, in patients with ventilatory insufficiency, this limit can be considerably lower as manifested by the dyspneic patient who has to rest every so often to "catch" his breath.

Although the amount of respiratory work is determined by the mechanical behavior of the lung-thorax system, the work performance depends upon the proper function of the ventilatory drive (23). Because the lungs follow the movement of the chest bellows passively, their role in the production of an adequate ventilatory drive is limited to the emission of adequate stimuli to the respiratory center. Although some of those stimuli originate in the periphery (shock, pain, cold, et cetera), the most potent stimuli are represented by changes in pCO_2, pH, and alveolar oxygen pressure (pO_2). It has recently been pointed out that jugular venous pCO_2 reflects more closely the respiratory stimulus than either alveolar or arterial pCO_2 (24). The afferent ventilatory stimuli are transmitted and coordinated in the respiratory center, which sends out the impulses initiating the contraction of the respiratory muscles. The amount of muscular contraction is a function of the strength of the stimulus, and the initial length and the physicochemical structure of the muscle fiber.

An insufficient ventilatory drive may lead to alveolar hypoventilation in spite of normal lungs. Such an insufficiency may occur either because of abnormal chest bellows, neuromuscular disorders, derangement of the respiratory center, or deficient ventilatory stimuli. Thus, though the general aspects of ventilatory mechanics can be represented by a relatively simple picture, the over-all ventilatory performance is complicated by the variation of the ventilatory drive. Much work remains to be done in order to clarify these relationships.

Summary

The working capacity of the chronic pulmonary patient is often limited by his inability to perform the respiratory work necessary to ensure proper gas exchange across the alveolo-capillary membrane. The factors responsible for an increase in the work of breathing are discussed and the various methods of estimating the metabolic costs of breathing are reviewed. The proper application of mechanical ventilatory devices depends upon the physical properties of the lung thorax system. Only if the latter are taken into account can proper ventilation with minimal pressure gradients be achieved. Finally, the relations between respiratory work and respiratory acidosis are briefly discussed, and the importance of a proper ventilatory drive for effective ventilation is stressed.

Sumario

La Mecánica de la Respiración. II. La Labor Respiratoria

La capacidad para el trabajo del enfermo pulmonar crónico se ve a menudo limitada por su

incapacidad para ejecutar la labor respiratoria necesaria para asegurar el debido intercambio de gases a través de la membrana alvéolocapilar. Se discuten los factores que intervienen en el aumento de la labor respiratoria y se repasan los varios métodos empleados para calcular los costos metabólicos de la respiración. La adecuada aplicación de los aparatos de ventilación mecánica depende de las propiedades físicas del sistema toracopulmonar. Unicamente tomando esto en cuenta cabe obtener la debida ventilación con las pendientes mínimas de presión. Al final, se discute brevemente la relación entre la labor respiratoria y la acidosis respiratoria, recalcándose la importancia que reviste un impulso ventilador adecuado para obtener ventilación eficaz.

Resume

La mécanique de la respiration. II. Le travail respiratoire

La capacité de travail du patient atteint de maladie pulmonaire chronique est souvent limitée par son incapacité d'exécuter le travail respiratoire nécessaire à l'échange adéquat des gaz à travers la membrane alvéolo-capillaire. Les facteurs responsables de l'accroissement du travail respiratoire sont étudiés et les diverses méthodes d'évaluation de la dépense énergétique entraînée par la respiration sont passés en revue. L'application appropriée d'appareils ventilatoires mécaniques dépend des propriétés physiques du système thoraco-pulmonaire. Ce n'est que lorsqu'il en est tenu compte qu'il est possible d'obtenir une ventilation adéquate avec des gradients de pression minima. Enfin, la corrélation entre le travail respiratoire et l'acidose respiratoire est brièvement commentée. Les auteurs insistent sur l'importance d'une dynamique ventilatoire appropriée pour que la ventilation soit efficace.

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MECHANICS OF BREATHING[1]

III. The Influence of Ventilatory Mechanics upon Gas Exchange and Pulmonary Circulation

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(Received for publication September 12, 1958)

Introduction

The independent investigation of circulatory hemodynamics and pulmonary function has unfortunately led to an unjustified separation of circulatory and ventilatory mechanics. Although this may be more practical in the field of clinical investigation, particularly in the preoperative evaluation of thoracic surgery, it fails to relate two functions which are designed essentially for the same purpose, namely, adequate oxygenation and decarbondioxidation of the body tissues. Cardiopulmonary physiology should be studied as a whole, and the contacts between the hemodynamic physiologist and the pulmonary physiologist should not be limited to polite greetings in the hospital corridor.

Major contributions in the relations of pulmonary ventilation and circulation were made in the late nineteenth and early twentieth centuries (1, 2). The demonstration of a clinically applicable technique of cardiac catheterization by Cournand and his associates made experimental verification of the theories and *in vitro* experiments of the older writers possible (3–15).

Respiratory Variations in Stroke Volume and Vascular Resistance

The ventilatory activity of the thorax can be considered as an additional pump. The greater negativity of intrathoracic pressure during inspiration enhances venous return, thereby increasing the stroke volume of the right ventricle. This leads to a larger filling of the lesser circulation followed by an increased filling of the left heart and a rise in left ventricular stroke volume. During periods of decreased intrathoracic negativity (expiration, positive pressure breathing), the reverse occurs. The impedance of the pulmonary circulation introduces a phase lag in the response of the right and left ventricles. This may lead to a decrease in the stroke volume of the left ventricle during inspiration. As the respiratory rate increases, the phase lag increases and the stroke volume variation decreases.

The resistance and capacity of the pulmonary vascular bed depend upon the lung volume. With increasing lung volume, the vascular capacity increases first and then starts to decrease (11). As a corollary, pulmonary vascular resistance decreases first and then rises (12, 13). The degree of inflation at which the vascular resistance begins to rise depends primarily upon the capacity of the vascular bed. In conditions in which the vascular bed is reduced (pneumonectomy, chronic pulmonary emphysema), pulmonary vascular resistance increases at much lower lung volumes than in the normal subject.

The pulmonary vasculature is a complex distensible system, which has a vessel caliber that increases as transmural pressure rises. Flow resistance decreases in proportion to the fourth power of the radius. Therefore, pulmonary vascular resistance decreases as either pulmonary arterial pressure or left atrial pressure increases until a plateau is reached, where further pressure changes are without effect upon pulmonary vascular resistance (13).

Transmission of Intrathoracic Pressures

The transmission of respiratory intrathoracic pressure variations to all elastic intrathoracic organs constitutes the base for the substitution of intra-esophageal pressures for intrapleural pressures. The recorded pressure in a given organ, however, will deviate from the actual intrathoracic pressure by a certain amount, which is determined primarily by the pressure volume characteristic of the wall of that particular organ (14). If in the wake of a change in ventilatory mechanics, the intrapleural pressure rises, the pulmonary vascular diastolic pressure may show either a greater, a similar, or a smaller rise. If the intrapleural and the intravascular pressure rises

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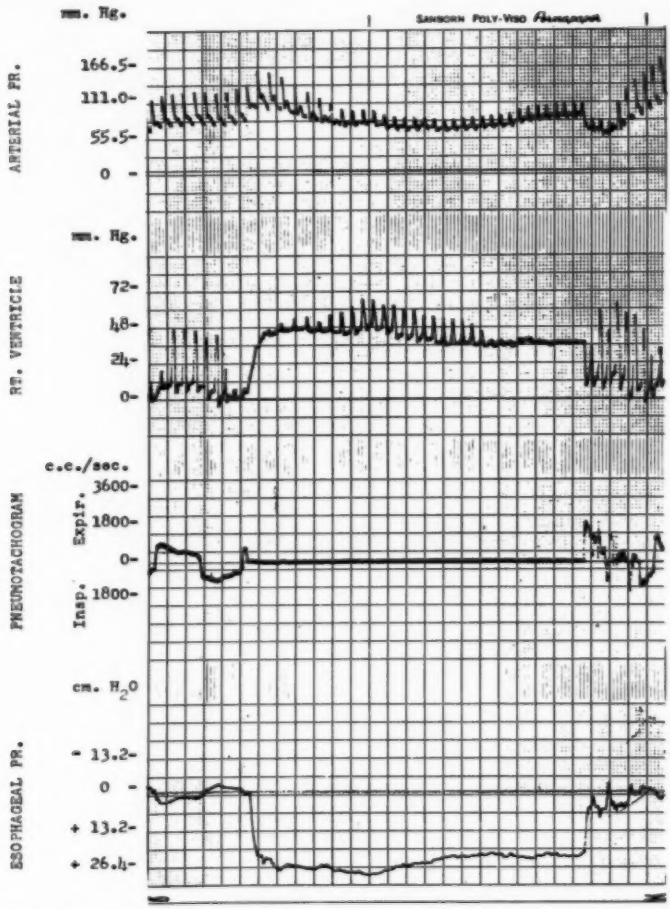

FIG. 1. Circulatory effects of increased intrathoracic pressure (Valsalva) in patient with chronic pulmonary emphysema.

are similar, it may be concluded that the vascular pressure rise is probably the effect of passive transmission of intrathoracic pressure. However, if the vascular pressure rise exceeds the intrathoracic pressure rise (figure 1), a change in the physical characteristics of the vessel must have occurred, due either to increased filling or to a change in vascular tone. Any rise in vascular diastolic pressure must be associated with either an increase in blood volume or in vascular tone, or both. Under these conditions, the transmission or respiratory intrathoracic pressure changes into the vasculature would be impaired and the vascular pressures would vary less between inspiration and expiration than before the rise. However, an increase of respiratory vascular pressure variations in the vasculature is quite frequently associated with a rise in diastolic pressure. This can only be explained by either rhythmic changes in stroke volume (vascular filling) or in vascular tone synchronous with respiration. Dubois and Marshall (15) have pointed out that pulmonary capillary flow during a normal respiratory cycle is relatively constant due to a compensation of increased inspiratory right ventricular stroke volume by an increase in pulmonary vascular

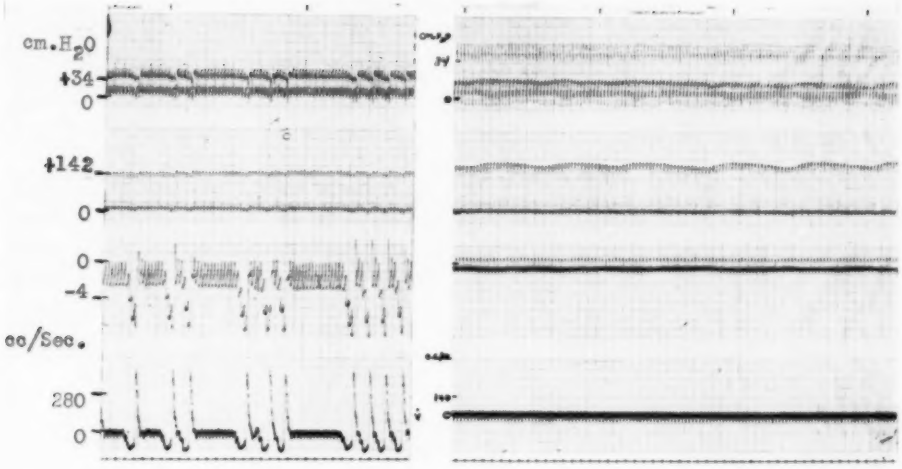

FIG. 2. Circulatory dynamics during normal breathing (at left) and during diffusion respiration (at right) of male dog, 11 kg. *Tracing from top to bottom:* right ventricular pressure, left ventricular pressure, transpulmonary pressure, and flow.

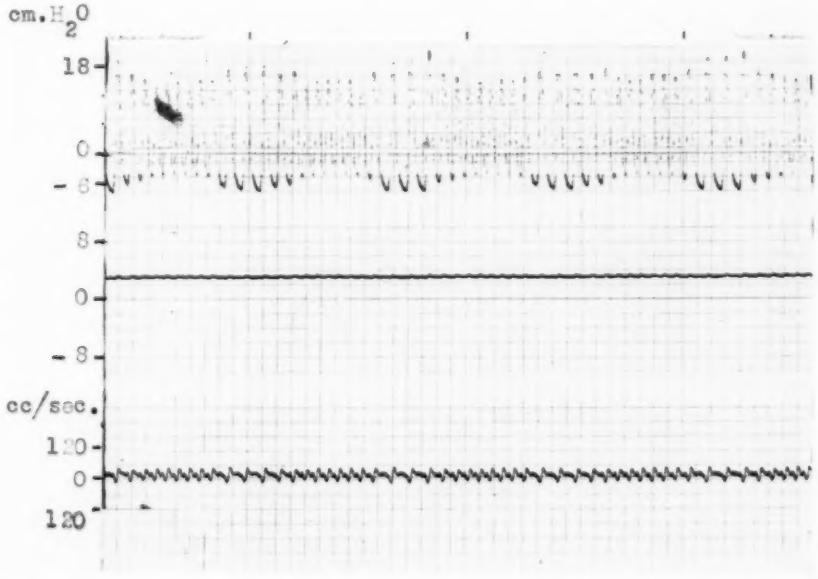

FIG. 3. Diffusion respiration: male dog, 25 lb. (*Note:* Sinus arrhythmia and "resp." pressure variations of right ventricle despite absence of intrathoracic pressure variations. Paper speed 10 mm. per second.) *Tracing from top to bottom:* right ventricular pressure, intra-esophageal pressure, and airflow.

resistance. However, present knowledge is inadequate to differentiate between vascular changes, due to variations in filling, and those due to active vasomotion.

In general, respiratory pressure variations in different segments of the pulmonary vasculature are quite different. These pressure variations increase from the right heart to the left heart, reaching a maximum in the pulmonary capillary. This simply reflects the physical properties of the various vascular segments. The recorded vascular pressure change following a cardiopulmonary event may be smaller or larger than the intrapleural pressure change (14, 22). The variations in effective pressure may, therefore, be quite different from the intravascular pressures recorded against a fixed baseline. The correction for intrathoracic pressure variations, however, poses some problems. The most logical procedure seems to be to correct mean vascular pressure for mean intrathoracic pressure, all the mean values being integrated with respect to time. It is important to remember that effective or transmural vascular pressure, on the one hand, and wall tension of the vessel, on the other, determine the cross-section of the vessel according to the LaPlace formula.

Vascular resistance is defined as the ratio pressure gradient over cardiac output. The pressure gradient in the case of total pulmonary vascular resistance represents the difference between mean pulmonary arterial and mean left auricular pressure. This pressure gradient has nothing to do with transmural pressure. Thus, the latter does not enter into the calculation of pulmonary vascular resistance, but plays an important role in its evaluation. If an increase in pulmonary vascular resistance is associated with a decrease in transmural pressure, at least part of the resistance increase can be attributed to the lowering of the cross-sectional area of the vasculature, and vice versa. Thus, the estimation of changes in intrathoracic pressure becomes important for the evaluation of changes in pulmonary vascular resistance and not for its calculation.

The fact that intravascular respiratory pressure variations exceed intrapleural pressure variations can only be explained by invoking a central oscillating mechanism, modifying either stroke volume or vascular tone synchronously with respiration (16, 17). In figures 2 and 3 "respiratory" vascular pressure variations in the right and left ventricles in the absence of intrapleural pressure variations (diffusion respiration)[3] are shown. The latter have been proved to be the effect of irradiation (spill-over) from the respiratory center to the vasomotor center (17). They may (figure 3) or may not (figure 2) be associated with sinus arrhythmia.

The Influence of Oxygen and Carbon Dioxide upon Pulmonary Circulation

Total pulmonary insufficiency is characterized by a decrease in alveolar ventilation (18, 19). As a consequence, alveolar oxygen pressure (pO_2) is decreased and alveolar carbon dioxide pressure (pCO_2) is increased. Sooner or later, this leads to respiratory acidosis. Both the low alveolar pO_2 and the high alveolar pCO_2 result in pulmonary hypertension. The constriction of the pulmonary vascular bed increases pulmonary vascular resistance. The chronic cor pulmonale in chronic pulmonary emphysema is partly the result of this mechanism (19) and partly due to an increase in the work of breathing. If either the alveolar pO_2 is increased by oxygen therapy and or/ the alveolar pCO_2, in addition, is decreased by artificial ventilation, the pulmonary vascular resistance and pulmonary arterial pressure decreases and the cardiac output increases. This type of pulmonary hypertension (functional constriction of the vascular bed) has to be clearly distinguished from other forms of pulmonary hypertension in which anatomic changes are not influenced by the improvement of alveolar ventiation. That the vasoconstriction in the wake of hypoxia and hypercapnia may not be restricted to the arterioles but may affect the venules has been suggested by Nisell (20).

Lung Volume and Diffusion Capacity of the Lungs

In general, the diffusion capacity of the lungs increases with lung volume. This is to be expected

[3] Diffusion respiration can be described as a state in which oxygenation of the blood occurs in spite of the absence of respiratory movement. The subject is first denitrogenized in an environment of pure oxygen. His respiratory movements are then arrested either by narcosis or by curare. The oxygen uptake by the pulmonary blood flow then creates a negative pressure within the lungs, thereby replacing the used oxygen from the pure oxygen environment. Obviously, the procedure is limited by the rapid accumulation of carbon dioxide which cannot be eliminated.

FIG. 4. E.C., 28-year-old female. Esophageal pressure during breath-holding in a patient with congestive heart failure (supine position). *Tracing from top to bottom:* Tidal volume, esophageal pressure, and flow rates.

because a larger lung volume represents a larger diffusing area (21). However, from the relationship between lung volume and capacity of the pulmonary vascular bed, it is to be expected that an optimal lung volume exists when the diffusion capacity is maximal. On either side, the diffusion capacity would be expected to decrease. The situation is complicated, however, by the variation of alveolar ventilation and blood flow with changes in lung volume. It is generally admitted that the present techniques of measuring diffusing capacity in the presence of unequal ventilation are not very reliable.

THE INFLUENCE OF CIRCULATION UPON VENTILATION

The heart action provokes rhythmic changes in intrathoracic pressures and, consequently, movement of air in and out of the thorax (figures 2, 3, 4). These airflow rates are produced by differences in instantaneous thoracic inflow and outflow of blood, i.e., the difference between stroke volume of the left ventricle and the venous return into the right atrium at a given movement. Cardiac systole is associated with inspiratory flow and diastole with expiratory flow. Changes in cardiac activity lead also to changes in the airflow rates caused by the cardiac cycle. Although the volume of air exchange due to cardiac activity is physiologically insignificant, it may become of some importance in patients with severe hypoventilation by promoting better distribution of inspired air.

SUMMARY

The pulmonary vasculature is a distensible, low-pressure system, subjected to cyclic variations in pressure and volume because of the respiratory movements. The effective (transmural) pressure in a thoracic vessel represents the pressure difference between the inside and the outside and, together with the wall tension, determines the vessel size. The influence of the respiratory movements upon cardiac stroke volume and vascular resistance are discussed, and the effects of oxygen and carbon dioxide upon the pulmonary circulation are briefly reviewed.

Sumario

La Mecánica de la Respiración. III. El Influjo de la Mecánica Ventiladora sobre el Intercambio de Gases y la Función Pulmonar

La vasculatura pulmonar constituye un sistema distendible de presión baja, expuesto, por virtud de los movimientos respiratorios, a variaciones cíclicas de la presión y del volumen. En un vaso torácico, la presión eficaz (transparietal) representa la diferencia en presión entre el interior y el exterior y, unida a la presión parietal, determina el tamaño del vaso. Se discute el influjo de los movimientos respiratorios sobre el volumen del latido cardíaco y la resistencia vascular, repasándose sucintamente los efectos del oxígeno y del bióxido de carbono sobre la circulación pulmonar.

Resume

La mécanique de la respiration. III. L'influence du mécanisme ventilatoire sur l'échange des gaz et la fonction pulmonaire

Le système vasculaire pulmonaire est distensible, c'est un système à basse pression soumis à des variations cycliques de pression et de volume causées par les mouvements respiratoires. La pression efficace (transmurale) dans un vaisseau pulmonaire représente la différence de pression entre l'intérieur et l'extérieur et détermine le calibre du vaisseau ainsi que la tension de la paroi. L'influence des mouvements respiratoires sur le volume de l'impulsion cardiaque et la résistance vasculaire sont commentés; les effets de l'oxygène et du CO^2 sur la circulation pulmonaire sont brièvement passés en revue.

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IS A VOCATIONAL REHABILITATION PROGRAM JUSTIFIED IN A TUBERCULOSIS HOSPITAL?[1]

ARTHUR J. MARION AND DAVID SALKIN

(Received for publication November 3, 1958)

INTRODUCTION

Most tuberculosis hospitals provide some measure of assistance to patients who need vocational rehabilitation. Because of the cost of such programs, private hospitals are usually restricted to a limited occupational therapy facility. At the other end of the scale, many Veterans Administration hospitals have and are providing extensive rehabilitation programs. One of the most extensive of these is in operation at the San Fernando Veterans Administration Hospital.

Justification for the additional cost in time and money of rehabilitation programs in tuberculosis hospitals has been taken for granted, or based on a small, informally recorded sample of "successes." A review of the literature over the past five years has revealed only one clear-cut demonstration of the benefits of a rehabilitation program.

In a follow-up study five years after hospital discharge, Warren (1) compared 79 participants in a post-hospital rehabilitation program with 161 nonparticipants. Warren's two groups were remarkably similar in terms of a great variety of pre-hospital variables. Five years after hospital discharge the nonparticipating group, as compared with the participants in rehabilitation, contained half as many employed, three times as many unemployed, twice as many with relapsed disease, and three times as many who had died. Expenditures of public funds, both for relief payments and for further hospitalization, were markedly lower for the group of rehabilitation participants.

At the San Fernando Veterans Administration Hospital, patients who need vocational rehabilitation are able to take advantage of a broad range of services designed to help them choose and prepare for full-time training or work immediately following hospital discharge. Under the direction of a professionally trained psychologist (Ph.D. in counseling psychology), the Vocational Counseling Service evaluates the vocational handicap, if any, of all patients entering the hospital. Those in need of vocational rehabilitation are offered the opportunity to begin the process of testing, counseling, and occupational exploration to enable them to plan their vocational future.

As these plans evolve for each patient, he is able to test or modify his objectives and begin preliminary phases of training and develop initial skills in the various clinics of the Physical Medicine and Rehabilitation Service. Regular hours are scheduled for any necessary education and occupational and manual arts therapy to further his aims. When he reaches an ambulatory status, he enters a graduated work therapy program in which his work tolerance is increased to a final eight hours daily in a job that prepares him further for his post-hospital vocation. Effective collaboration with appropriate community agencies provides job or training placement if needed by the patient at the time he has completed the rehabilitation program and is ready for discharge.

The participation of the patient in these rehabilitation activities has tended to extend his hospitalization somewhat beyond that of the patient who was not in need of vocational rehabilitation or who chose to rehabilitate himself at home. Because of the current contentions (in some quarters (2, 3)) regarding the value of such rehabilitation programs, a study was begun to answer this question: Can the rehabilitation program with its concomitant extension of hospital treatment be justified in the more rapid achievement of full-time work or training status, less unemployment, and fewer job changes?

METHOD

The outpatient clinic at the San Fernando Veterans Administration Hospital serves approximately 1,000 veterans with service-connected disability compensation for tuber-

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culosis. The majority of these outpatients had been hospitalized for their disability at this hospital. Because of the relatively recent advances in both medical therapy and rehabilitation at the hospital, it was decided that the sample for the follow-up study would be selected only from those veterans who had been hospitalized at San Fernando since January 1, 1953, for at least ninety days.

Prior to the patients' return for their periodic examinations in the outpatient clinic, their hospital records were analyzed to select those who met the criteria for length of hospitalization during the chosen period. The patients selected were scheduled for a structured interview. The objective of the interview was to obtain relatively complete information from the patient regarding his medical, educational, vocational, military, marital, and family history with his period of hospitalization. This history was divided into pre-hospital and post-hospital segments. In addition, the patient's account of his hospitalization experiences and their value to him was recorded.

On the basis of the information gathered, the patients interviewed were placed in one of four groups for analysis of the data. First, a division was made on the basis of "need for vocational rehabilitation." Patients who had returned following hospitalization to the identical job and place of employment they were in prior to hospitalization were considered not to have been in need of vocational rehabilitation. The data from this group were analyzed separately. The remainder of the subjects in this study was divided again into two groups—those who received regular discharges, and those who received premature discharges from the hospital. Obviously, those with premature discharges did not complete the rehabilitation program. Indeed, most of them did not enter into it. Finally, those patients who received regular discharges were again divided into two groups—those who received their discharges from the rehabilitation ward and, therefore, completed the rehabilitation program; and those who received their discharges from other wards and, therefore, did not participate in the full rehabilitation program.

The interviewing was done during the period from October 15, 1957, through February 1, 1958. Data were collected on 165 subjects. The classification of subjects according to the rationale above is indicated in table 1. The basic plan was to compare each of these groups in terms of rapidity of vocational readjustment, job stability, occupational level, and satisfaction. If the rehabilitation group surpassed the other groups in these characteristics, the difference could be attributed to the rehabilitation program, provided that the groups did not differ in any other relevant parameters. In order to assess the influence of such parameters, the groups were compared on a variety of dimensions that were considered potentially relevant.

TABLE 1

CLASSIFICATION OF PATIENTS ACCORDING TO THEIR VOCATIONAL REHABILITATION ASPECTS

Group Classification	Number
Rehabilitation	65
Non-rehabilitation	43
Premature discharge	34
Non-need rehabilitation	23
Total	165

How the four groups compare in these respects is shown in table 2. Compared with one or more of the other groups, the "rehabilitation group" contained younger men, fewer married men, fewer "far advanced" cases, men with longer hospitalization records, and men with fewer jobs and less unemployment. The age factor was correlated with each of these other variables with the exception of "longer hospitalization." With time held as a constant, only the "non-need" group showed significantly fewer jobs and significantly less unemployment.

RESULTS

The measures on which the four groups were compared in the post-hospital period included post-hospital education, post-hospital occupational level, number of jobs, months unemployed, time from hospital discharge to any job or training, and time from hospital discharge to full-time job or training. The group comparisons on these variables are indicated in table 3.

The patients in the rehabilitation group improved their formal *educational level* by an average of 1.1 years. Although their pre-discharge educational advantage over the non-rehabilitation and premature discharge groups was not statistically significant, the post-hospital increase in educational achievement of the rehabilitation group produced a significant difference. This is one concrete demonstration of the use made of the rehabilitation program by patients who com-

TABLE 2

DIFFERENCES AMONG THE CRITERION GROUPS IN POTENTIALLY RELEVANT PRE-DISCHARGE VARIABLES

Variable	Group			
	Rehabilitation	Non-Rehabilitation	Premature Discharge	Non-need Rehabilitation
Age (mean)	34.1	43.3*	38.8*	41.0*
Married (%)	57.0	79.0*	68.0*	91.0*
Number of dependents (mean)	1.7	1.7	1.9	2.2
Far advanced tuberculosis (%)	44.0	60.0*	47.0	35.0
Total months in hospital (mean)	39.0	41.0	26.0	22.0
Number of months of last hospitalization (mean)	21.0	13.0*	8.0*	14.0*
Education (mean years)	11.3	10.7	10.6	12.8
Number of jobs (mean)	5.4	8.3	7.0	5.2
Occupational level (mean)†	7.2	8.6*	7.7	10.8
Number of months unemployed (mean)	16.3	67.0*	36.5	11.5

* Significant at 0.05 level of confidence in reference to the rehabilitation group.

† For this study occupational level was defined and measured as the summed rating of the general and specific training time required for the occupation held. These ratings were compiled by the Bureau of Employment Security, U. S. Employment Service, in the publication *Worker Trait Requirements* (U. S. Government Printing Office, Washington, D. C.).

pleted it. Many began to add to their educational achievement while in the hospital, and an advanced educational program often became a major part of the individual's rehabilitation plans.

The rehabilitation group also demonstrated a gain in *occupational level* of 1.1 scale points, whereas the largest gain among the other three groups was only 0.2 scale points. The non-rehabilitation group actually lost 0.4 scale points. Their loss and the rehabilitation group gain in the post-hospital compared with the pre-hospital periods eliminated the statistically significant advantage on this variable held by the non-rehabilitation group in the pre-hospital period.

The post-hospital periods of *unemployment* among the four criterion groups include the time taken to get the first job following discharge from the hospital. As expected, the non-need group were employed most quickly and have worked most steadily since they left the hospital. The rehabilitation group had significantly less unemployment than did the non-rehabilitation group and the premature discharge group. Most of the difference in unemployment was produced by the varying success each group had in finding the first job or training opportunity. The time between hospital discharge and the interview for this study did not differ significantly among the four groups. This interval varied from a mean of twenty-three months for the rehabilitation group and a mean of twenty-six months for the non-need group to a mean of twenty-eight months for both the non-rehabilitation and the premature discharge groups.

As implied in the results regarding post-hospital unemployment, the non-need group took less time on the average to *return to work*. Surprisingly, this took an average of seven weeks from the date of their discharge, and the most frequent reason for the delay in returning to work was the "need for a vacation." The time taken by the non-need group to return to work was significantly less than the time taken by the rehabilitation group. However, with the criterion of time taken to reach *full-time work* or training, the non-need group did not differ significantly from the rehabilitation group. Comparison of the rehabilitation group with the non-rehabilitation and the premature discharge groups reveals a highly significant difference in favor of the

TABLE 3

DIFFERENCES AMONG THE CRITERION GROUPS ON POST-HOSPITAL VARIABLES

Variable	Group			
	Rehabilitation	Non-Rehabilitation	Irregular Discharge	Non-need Rehabilitation
Education (mean years)	12.4	11.1*	10.8*	12.8
Occupational level	8.3	8.2	7.9	11.0*
Number of jobs (mean)	1.4	0.9	1.3	1.1
Number of months unemployed (mean)	5.9	14.9*	16.8*	1.5*
Number of weeks to any job or training (mean)	19.3	60.0*	61.7*	6.9*
Number of weeks to full-time job or training (mean)	27.6	73.5*	75.2*	22.6

* Significant at 0.05 level of confidence in reference to the rehabilitation group.

TABLE 4

FREQUENCY DISTRIBUTION OF TIME FROM HOSPITAL DISCHARGE TO ANY WORK OR TRAINING (FULL- OR PART-TIME)

Time in Weeks to Work or Training	Group			
	Rehabilitation 65 Patients	Non-rehabilitation 43 Patients	Premature Discharge 34 Patients	Non-need Rehabilitation 23 Patients
0 to 4	33	5	2	13
5 to 20	12	5	1	7
21 to 40	9	5	6	3
41 to 150	4	9	12	0
Not yet working	7	19	13	0

TABLE 5

FREQUENCY DISTRIBUTION OF THE POST-HOSPITAL VOCATIONAL - EDUCATIONAL ACTIVITIES OF MATCHED "REHABILITATED" AND "NON-REHABILITATED" PATIENTS

Group	Total	In Training	Part-time Work	Full-time Work	Not Yet in Work or Training
Matched rehabilitation	33	6	5	17	4
Matched non-rehabilitation	33	12	5	5	11

rehabilitation group in terms of time taken to get any job or training and to reach full-time work or training.

Because of the unbalanced nature of the distribution of times from discharge to work, the means of the groups may not be as representative as medians or frequency tables. In addition, the use of means as a summary statistic is further complicated by the patients in each group who had not yet gone to work at the time of the follow-up interview. In the use of the mean as a group comparison, it was decided to include those who had not found employment and use the length of time since their discharge as the "time to work or training." This problem does not arise when a frequency tabulation is used.

The data regarding time from hospital discharge to first post-hospital job or training is represented in table 4 in such form. The advantage of the rehabilitation group in rapid return to employment or training as compared with the non-rehabilitation and premature discharge groups is even more obvious here than in the comparison of means. The record of the rehabilitation group is remarkably similar to that of the non-need group, whose jobs were awaiting them following their hospital discharge. This may reflect the fact that most of the rehabilitation group takes advantage of the contacts, time, and vocational counseling available in the rehabilitation program. Such patients often have a job or training opportunity already arranged and waiting at the time of their discharge.

Matched Grouping

In addition to the opportunities available to the rehabilitation group in choosing and making concrete arrangements for work or training while completing the rehabilitation program, the fact that this group was composed of younger men with less extensive disease may have biased the results in their favor. In order to test this hypothesis two *matched groups* were selected from the original groups called the rehabilitation group and the non-rehabilitation group. The matched groups were selected, pair by pair, with identical variables of age and extent of disease at the time of discharge for each pair. It was thus possible to select 33 matched pairs and compare these two matched groups on the same criteria as those used to compare the other groups. Some of the frequency data regarding these two matched groups are shown in table 5. The matching process produced a mean age of 38.18 years for both groups and such tuberculosis extent that each group contained 18 far advanced, 11 moderately advanced, and 4 minimal cases.

The fewer rehabilitation group members still

TABLE 6

FREQUENCY DISTRIBUTION OF TIME FROM HOSPITAL DISCHARGE TO ANY WORK OR TRAINING FOR MATCHED GROUPS OF REHABILITATED AND NON-REHABILITATED PATIENTS

Group	Total	Weeks				
		0-4	5-20	21-40	41-150	Not Yet Working
Matched rehabilitation	33	13	9	5	2	4
Matched non-rehabilitation	33	5	5	4	9	11

TABLE 7

DIFFERENCES BETWEEN MATCHED GROUPS OF REHABILITATED AND NON-REHABILITATED PATIENTS ON VARIABLES RELATED TO POST-HOSPITAL VOCATIONAL ADJUSTMENT

Group	Mean Months Since Discharge	Mean Months Unemployed	Mean Weeks to Job or Training	Mean Weeks to Full Job or Training
Matched rehabilitation	27.2	8.1	26.0	37.4
Matched non-rehabilitation	25.2	10.4	39.3	52.2

unemployed at the time of the follow-up study and the greater number in full-time work demonstrate an apparent value in the rehabilitation program even when the typically greater youthfulness and less extensive disease of the usual rehabilitation group are eliminated as independent variables. The time taken by members of each matched group to begin a job or training, as well as the rapidity of readjustment of the rehabilitation group, are shown in table 6.

These differences reveal themselves in the statistical tests of differences of the means as well, although not as graphically as tables 4 and 5. These differences are indicated in table 7. Because of the relatively small sample and the wide variance on each of these variables, none of the differences in table 7 is significant at the 5 per cent level of confidence.

DISCUSSION

The evidence presented in this study appears to justify the additional cost and lengthened hospitalization of the rehabilitation program. Even with the use of matched groups, one may contend that the motivation of the patients to volunteer in such a program is in itself a method of preselection. It may be argued that the superior post-hospital adjustment of the rehabilitation group is due to such motivation (and the associated personality characteristics) rather than to the experience obtained in such a program. To eliminate this variable of self-selection, one would have to select only patients who express a desire for the rehabilitation services and then assign them randomly to an experimental group and a control group. However, such a method which would deny rehabilitation to half of the total research group (despite their desire and need for it) would be most difficult to perform and would be neither legally nor ethically defensible.

The alternative would be to allow voluntary self-selection to operate, and assume or demonstrate that the personality factors involved have an unsystematic or random influence upon the dependent variable of post-hospital adjustment. In the present study, such an assumption was made on the basis of the random distribution of personality traits, work attitudes, and level of aspiration among subjects of all of the groups. This assumption was not tested, however.

The present conclusion that the rehabilitation program is justified is, therefore, qualified with the recommendation that there is need of an additional study in which the experimental controls are developed prior to the selection of the patients and prior to their participation or nonparticipation in the rehabilitation program.

SUMMARY

In order to determine whether the rehabilitation program was justified at this tuberculosis hospital, 165 outpatients were studied intensively from the standpoint of their medical, educational, vocational, and marital histories during their pre-hospital, hospital, and post-hospital periods.

The patients were classified into four groups: a rehabilitation group, a non-rehabilitation group, a premature discharge group, and a "non-need" group consisting of those who returned to their exact pre-hospital jobs.

On the basis of post-hospital adjustment, the rehabilitation group surpassed the non-rehabilitation and premature discharge groups in less unemployment, greater educational gain, greater occupational level gain, shorter time to get into a job or training, and shorter time to full-time work or training. An additional comparison of 33 matched pairs of subjects from the rehabilitation and non-rehabilitation groups demonstrated again a better post-hospital vocational adjustment by the rehabilitation group.

It is believed, therefore, that the described rehabilitation program is vocationally justified.

SUMARIO

¿Está Justificado un Plan de Rehabilitación Profesional en un Hospital de Tuberculosis?

A fin de determinar si estaba justificado un plan de rehabilitación en el hospital para tuberculosos de los AA., se estudió intensamente a 119

enfermos desde el punto de vista de sus historias médicas, pedagógicas, profesionales y conyugales durante los períodos prehospitalario, hospitalario y posthospitalario.

Los enfermos fueron clasificados en cuatro grupos: grupo de rehabilitación, grupo sin rehabilitación, grupo de alta inaprobada compuesto de los que abanadonaron el hospital prematuramente y grupo "sin necesidad," compuesto de los que volvieron a los puestos que ocupaban antes de ingresar en el hospital.

A base del ajuste posthospitalario, el grupo de rehabilitación sobrepujó a los grupos sin rehabilitación y de alta inaprobada en mostrar menos falta de empleo, mayor adelanto pedagógico, mayor elevación del nivel profesional, menor tiempo en tomar colocación o preparación y tardar menos en encontrar trabajo o adiestramiento de a tiempo completo. Otra comparación de 33 pares de sujetos equiparados tomados de los grupos de rehabilitación y sin rehabilitación demostró de nuevo mejor ajuste profesional por el grupo de rehabilitación después de la estancia en el hospital.

Parece, por consiguiente, que está justificado profesionalmente el plan de rehabilitación aquí descrito.

Resume

Un programme de réadaptation professionnelle est-il justifié dans un hôpital de la tuberculose?

De manière à déterminer si dans cet hôpital de la tuberculose un programme de réadaptation était justifié, 165 malades de dispensaires ont été étudiés de façon complète en ce qui concernait leurs antécédents: (médicaux, éducation, profession, vie maritale), durant les périodes préhospitalières, hospitalières et post-hospitalières.

Les malades ont été classifiés en quatre groupes: groupe de réadaptation, groupe sans réadaptation, groupe où les malades ont quitté prématurément l'hôpital contre l'avis du médecin, et groupe où la réadaptation n'était pas nécessaire, qui était composé de patients ayant repris exactement les occupations qu'ils avaient avant l'entrée à l'hôpital.

Sur la base de l'adaptation post-hospitalière le groupe de réadaptation surpassait le groupe sans réadaptation et celui des départs non-autorisés dans les conditions suivantes: moins de chômage, plus de connaissances acquises, niveau d'occupations plus élevé, réduction du temps nécessaire pour obtenir un emploi ou de la période d'apprentissage, période plus courte pour s'adapter à un travail ou un apprentissage à temps complet. Une étude comparée supplémentaire sur 33 paires de sujets appareillés venant des groupes de réadaptation et de non-réadaptation a démontré à nouveau la supériorité de l'adaptation professionnelle post-hospitalière du groupe de réadaptation.

En conséquence, les auteurs estiment que le programme de réadaptation décrit est justifié du point de vue professionnel.

Acknowledgments

The authors gratefully acknowledge the aid given to them by Sherman T. Stenberg, M.D., for arranging interviews and for help in gathering post-hospital data, and to Wilfred L. Shaw, M.D., for his interest and aid in the collection of in-hospital data.

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Case Reports

ENDOBRONCHIAL HAMARTOMA[1]

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(Received for publication December 16, 1958)

The vast majority of endobronchial tumors are bronchogenic carcinomas, a small number of bronchial adenomas accounting for most of the remainder. It is the purpose of this case report to record an instance of endobronchial hamartoma.

Case Report

A 62-year-old male clerk complained of fever, mild anterior chest pain, and a nonproductive cough of two weeks' duration. The symptoms had developed following an upper respiratory infection. Bronchoscopy was performed at another hospital and revealed a "lobulated growth at the orifice of the anterior segment of the left upper lobe." Biopsy was reported to cause suspicion of carcinoma.

By the time of admission to the Veterans Administration Hospital, Bronx, New York, the patient's symptoms had subsided. There was no evidence of weight loss. Vital signs were normal. Except for some increase in the anteroposterior diameter of the chest, the physical examination was entirely within normal limits.

Fig. 1 (Left). Postero-anterior roentgenogram. Infiltration is present in the upper lobe with an apparent left hilar mass.

Fig. 2 (Right). Lateral tomogram. No definite mass is identified.

Laboratory studies revealed no abnormalities. Roentgenographic study of the chest disclosed considerable prominence of the structures at the root of the left lung and diffuse infiltration throughout all segments of the superior division of the left upper lobe (figures 1 and 2). Pleural reaction was noted in this region and at the anterior portion of the base of the lung. No definite mass was discernible on tomographic

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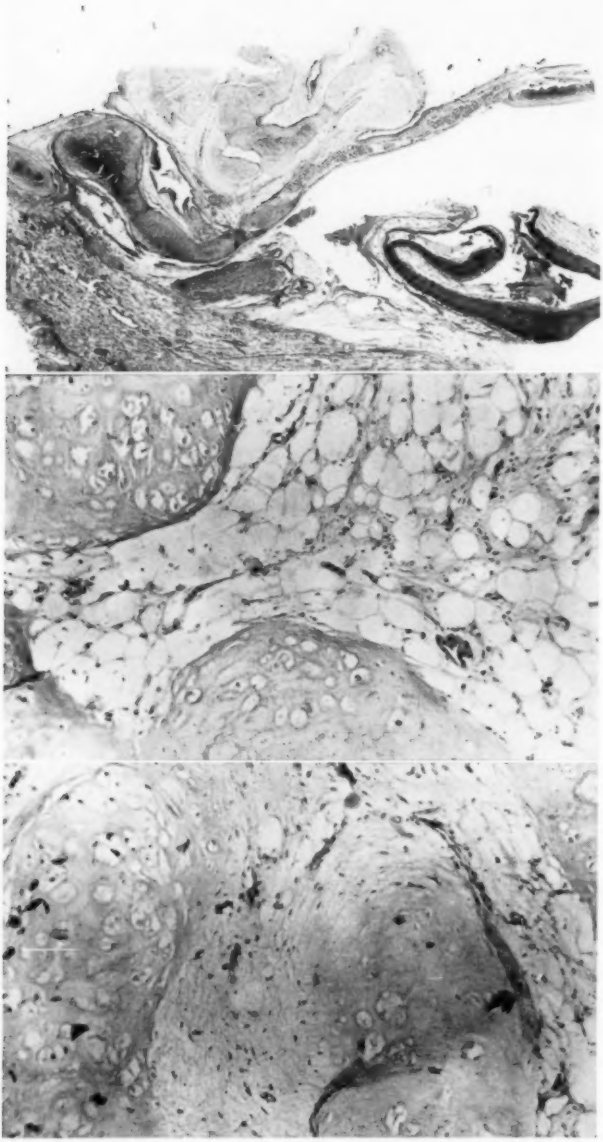

FIG. 3 (Top). Low-power (×7) view of endobronchial polypoid tumor mass. The tumor is covered by bronchial epithelium, and consists primarily of cartilage. Note the epithelium-lined spaces.
FIG. 4 (Center). Cartilage and fat. (×560)
FIG. 5 (Bottom). Apparent origin of cartilage from myxomatous connective tissue. (×560)

studies. Bronchoscopy revealed a sharp carina which was slightly deviated to the left. A small papillary growth was seen on the medial wall of the orifice of the left upper lobe bronchus. Biopsy was performed with difficulty because of the location of the lesion, and showed no abnormality.

Following therapy with penicillin and bronchodilators, roentgenograms showed some clearing of the left upper lobe infiltration. It seemed clear that the patient had an obstructing lesion of the left upper lobe bronchus, presumably a carcinoma, and that thoracotomy was indicated. At surgery a mass was palpated in the region of the left upper lobe orifice, and pneumonectomy was performed because of the clinical and histologic suspicion of carcinoma. The postoperative period was without untoward incident.

The specimen consisted of a left lung which was normal in size. No significant hilar adenopathy was found. The lower lobe was normal, and the upper lobe revealed chronic inflammatory changes macroscopically and microscopically. A small polypoid mass, measuring 1.1 by 0.7 by 0.6 cm., was present at the lower border of the left upper lobe orifice. It was lobulated, white, and relatively soft on section. The surface was smooth and glistening. The mass did not extend below the mucosa and, when not retracted, occluded the orifice to the upper lobe. Microscopic sections (figures 3–5) revealed that most of the lesion consisted of areas of adult cartilage. A few epithelium-lined glandular spaces were also present, as well as fatty and myxomatous connective tissue. The lesion was entirely covered with normal bronchial epithelium. The microscopic diagnosis was endobronchial hamartoma.

Discussion

The term hamartoma is used to describe a type of pathologic lesion which resembles both a *tumor*, on the one hand, and a congenital *malformation*, on the other. In fact, although some writers refer to them simply as "tumors," or as "tumors of developmental origin" (1), most prefer the term "tumor-like malformation." The clinical and pathologic characteristics of the lesions in different patients occasionally give support to belief in either the concept of neoplastic or congenital origin. For example, the histologic features per se are benign and show no evidence of increased mitotic activity. On the other hand, occasions have occurred in which proved hamartomas have increased in size with time (2, 3) or have not been present on roentgenograms taken in previous years (4). Ordinarily, the histologic structure reveals an abnormal mixture of the normal elements that comprise the organ in which the lesions are found. This abnormality of mixing may involve the quantity, arrangement, or degree of development of any of the elements of the parent organ.

When hamartomas involve the lung they are ordinarily composed mainly of hyaline cartilage, with varying degrees of fat, myxomatous connective tissue, bone formation, and cuboidal or columnar epithelium-lined clefts. The histologic variation between lesions is considerable and covers a broad spectrum in which some are almost purely of one histologic type, and are thus simply labelled "chondroma" or "cartilaginous hamartoma"; whereas others with greater histologic variability have been classified with compound names ranging from "adeno-chondroma" to "adeno-fibrolipo-chondromyxoma." Womack and associates (5) and others (6) even include bronchial adenoma in this group, although other writers believe that cogent evidence exists for not doing so. Most writers today use the term *hamartoma* when the lesions occur deep within the parenchyma of the lung; some preference still remains for the appellation *chondroma* when the localization is endobronchial.

Many reports (7–9) indicate that true chondromas are more frequent than endobronchial polypoid hamartomas; yet the Mayo group (10) has not seen one instance of a "pure" chondroma, and many of the lesions listed under this term in the literature (11, 12) contain numerous elements other than cartilage. This is probably begging the question, as Liebow (1, 9) believes that a certain amount of fat and connective tissue is not incompatible with the diagnosis of chondroma as opposed to hamartoma. It is beyond the scope of the present paper to review the evidence on this point, but it does appear clear that many cases previously described as "chondroma" were actually examples of what would today preferably be called "hamartoma"; and it is possible that all tumors within this broad range should be included in the same over-all category.

Hamartomas are relatively rare tumors, although the reported incidence is variable. Hodges (14), for example, calculated the autopsy prevalence in approximately 10,000 cases as 0.069 per cent; the incidence in the series of 8,000 autopsies of McDonald and associates (10) was 0.25 per cent, and in Rubin and Berkman's study of 8,800

autopsies (15), 0.32 per cent. The lesion has a much greater significance in clinical medicine, however, than these figures imply. Many solitary circumscribed lesions in asymptomatic people have been discovered in mass roentgenographic surveys and, because of the difficulty of differentiating carcinoma and various granulomas from these lesions by clinical and roentgenographic means (16–18), many hamartomas have been resected in recent years. The frequency of the lesion in this situation is revealed by the Mayo Clinic series (19), in which 16 per cent of all resected lesions of the solitary circumscribed type proved to be hamartomas. Johnston and Weinberg (20) reviewed 17 reported series of resections of "coin" lesions and found that 9.6 per cent of the resected nodules were hamartomas, representing 75 instances of the lesion.

Three different morphologic types of hamartoma have been described: *peripheral*, *endobronchial*, and *diffuse*. The latter, an extremely rare condition in which an entire lobe is replaced by multiple abnormal developmental foci, has been reported on only a few occasions (21, 22), mostly in children, and will not be mentioned again in this discussion. The occurrence of the peripheral form of hamartoma of the lung is well known to clinicians, radiologists, and surgeons, and is usually considered in the differential diagnosis of solitary nodules. However, that the lesions occur within the walls and lumina of major bronchi is not so well appreciated. This localization of hamartoma is quite rare, although the over-all specific incidence, both in relation to the general population and as a percentage of total hamartomas, is difficult to assess. In 1926, Hickey and Simpson (11) reviewed 40 cases of hamartomas reported in the literature up to that time; 4 of these were endobronchial. It is of interest that these writers preferred the term *chondroma* despite an excellent description of the histologic findings and discussion of the alternative *hamartoma*. Postlethwait and co-workers (23) found 8 additional cases in the literature, and added one of their own. This excludes Davidson's case (24), in which the tumor apparently consisted solely of cartilage. Also excluded are Cracovaner's 2 cases (25) for the same reason and the case of Weisel and associates (2), in which a large peripheral hamartoma appears to have entered the bronchus from without rather than to have originated within it.

TABLE 1

REPORTED CASES OF ENDOBRONCHIAL HAMARTOMA

Hickey and Simpson	(11)	1926	4*
Postlethwait *et al.*	(23)	1948	9*
Hall	(18)	1948	1
Carlsen and Kiaer	(8)	1950	1
Effler and Scheid	(6)	1951	1
Rubin and Berkman	(15)	1952	1
Sherrick	(29)	1952	1
Moersch *et al.*	(4)	1952	3
Brewer *et al.*	(30)	1953	1
Sutherland *et al.*	(31)	1953	2
Chardack and Waite	(32)	1953	1
Young *et al.*	(33)	1954	2
Store	(34)	1954	1
Chambers and Averill	(28)	1955	1
Paterson	(35)	1956	1
Adams	(36)	1957	1

* Including cases of other writers from previous years

Since 1948 an additional 18 cases have been reported in the British and American literature (table 1). No attempt has been made to review the foreign literature of this period. The present writer, therefore, was able to collect a total of 31 previous cases. Hodges (14) reviewed 33 cases of endobronchial hamartomas, including some chondromas, of a total of approximately 200 reported cases of hamartoma. This would represent an incidence of 16 per cent. In the review by Moersch and co-workers (4) of the Mayo Clinic *surgical* material, 3 of 35 cases were endobronchial, an incidence of 8.5 per cent. It should be recalled that in autopsy material this localization is much less common, none being noted in the Mayo cases (10), and only one of 28 being endobronchial in Rubin and Berkman's series (15). Endobronchial hamartoma is associated with symptoms, and therefore is much more likely to appear in surgical than in autopsy material; and when this localization is encountered the physician is so struck by the rarity of the lesion that often a case report ensues. For example, at the Bronx Veterans Administration Hospital 12 hamartomas of the lung have been encountered. All except the present case have been peripheral lesions, 3 of which have been reported in the radiologic literature (3). However, the present report of an endobronchial lesion is the first recording of any of these cases from a clinical service, and is limited to the presentation of the single instance of endobronchial localization.

The clinical picture of endobronchial hamartoma is that of any endobronchial lesion, and symptoms of obstructive pneumonitis develop. This is in marked contrast to the peripheral form, which unless strategically located so as to impinge upon a bronchus (2, 26, 27), is totally asymptomatic. Cough, sputum often productive of purulent material, fever, wheezing respirations, and pleural pain are frequent. Hemoptysis, often of considerable extent, has been reported on a number of occasions. The physical examination usually is not remarkable, diminished breath sounds over the affected lobe being the only abnormality. The diagnosis can be made at bronchoscopy or at thoracotomy.

In the patient described in the present report, the clinical and roentgenographic picture suggested an obstructing lesion in the left upper lobe bronchus. This was confirmed at bronchoscopy. Because a previous biopsy was reported as suspicious for carcinoma, a pneumonectomy was performed. It is possible that, if the true nature of the lesion had been appreciated, the resection could have been limited to a lobectomy.

Although hamartomas should be considered in the differential diagnosis of endobronchial obstructing lesions, their rarity as compared to bronchogenic carcinoma is such that it would be folly for the management of an individual patient to be affected by the knowledge of the existence of this benign diagnostic possibility. However, as these lesions have been successfully removed not only by lobectomy but also by other resectional procedures short of pneumonectomy, including bronchotomy at thoracotomy (28) and through the bronchoscope (4, 12, 18, 24, 25) on many occasions, it would appear to be wise for the surgeon to be certain of the histologic diagnosis of malignant neoplasm, if possible, prior to committing himself to a pneumonectomy.

Summary

An instance of an endobronchial hamartoma in a 62-year-old male has been recorded. This marks the thirty-second reported case of this uncommon lesion.

Sumario

Hamarcioma Endobronquial

Se describe un caso de hamarcioma endobronquial en un sujeto de 62 años de edad. Este constituye el trigésimosegundo caso descrito de esta rara lesión.

Resume

Hamartome endobronchique

Un cas d'hamartome endobronchique a été enregistré. Ceci représente le trente-deuxième cas de cette lésion rare ayant été rapporté.

Acknowledgment

The writer wishes to thank Dr. G. Eichner of the Department of Pathology for his helpful discussion of the pathologic aspects of this case.

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EVOLUTION OF PULMONARY SARCOIDOSIS AS REVEALED IN A CASE FOLLOWED OVER A SIX-YEAR PERIOD[1]

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(Received for publication December 29, 1958)

INTRODUCTION

The opportunity to study the morphologic changes in the lung during the course of such a complex disease as sarcoidosis rarely presents itself. Unless the disease process can be followed to the autopsy table, a veil of uncertainty exists and such a diagnosis can only be entertained after excluding other similar granulomatous conditions of known etiology. Hutchinson reported the first case of sarcoidosis in 1876 but it was not until 1889 that Boeck (1) gave a complete morphologic description of the disease.

The present case is one concerning a patient who was followed over a period of six years. Tissue taken from the lung for biopsy four and one-half years prior to death and necropsy tissues permit an appraisal of the disease process in the florid and terminal stages and contribute to the understanding of the evolution of sarcoidosis of the lungs.

CASE REPORT

This 47-year-old Negro male was first admitted to the City of Chicago Municipal Tuberculosis Sanitarium, in June, 1951. One month prior to admission a chest roentgenogram at an outpatient clinic had shown evidence of a diffuse disease process suggestive of tuberculosis. His first symptom was a cough which began about nine months before; subsequently, he noticed fatigue, night sweats, shortness of breath, weight loss, and fever. The patient smoked two packages of cigarettes daily and used alcoholic beverages sparingly.

Physical examination revealed a well-developed adult Negro male showing evidence of weight loss. He was afebrile. His blood pressure was 138 systolic, 80 diastolic in mm. of mercury; pulse was 100; and the configuration and size of the heart were normal. Examination of the chest revealed moist and coarse rales at both apices of the lungs. There were no other significant findings.

The initial chest roentgenogram (figure 1) revealed a granular infiltration disseminated throughout both lung fields, prominence of the right hilum, and blunting of both costophrenic sinuses. The erythrocyte count was 5,590,000 per cu. mm., and the hemoglobin was 73 per cent. The leukocyte count was 5,250 per cu. mm. with 75 per cent neutrophils, 15 per cent lymphocytes, and 10 per cent monocytes. A serologic test for syphilis (Kahn) was positive ("2 units"). There were no abnormal urinary findings. Repeated cultures and animal inoculations of the sputum and gastric contents were negative for tubercle bacilli and fungi. Skin tests using histoplasmin and coccidioidin were negative, and only the 1:10 dilution of Old Tuberculin evoked a positive reaction.

Because of the appearance of the chest roentgenogram, the patient was given dihydrostreptomycin and para-aminosalicylic acid. Bronchoscopic examination in April, 1952, was essentially normal. The nonprotein nitrogen was 44 mg. per 100 ml.; the total protein was 6.76 gm. per 100 ml., with 3.21 gm. per 100 ml. albumin and 3.08 gm. per 100 ml. globulin. Roentgenograms of both hands were negative for bone lesions. Because there was no appreciable change in the chest roentgenogram and a definitive diagnosis had not been established, tissue was taken from the lung for biopsy.

Microscopic sections of the lung contained noncaseating epithelioid tubercles with many giant cells both of the Langhans' and nonspecific types. There was marked fibrous tissue proliferation about the tubercles, and many histiocytes laden with anthracotic pigment were noted. The bronchioles were dilated and surrounded by fibrous tissue, and many nonfunctioning alveoli were noted. Bacteriologic examination of the lung tissue (culture and animal inoculation) was negative. The diagnosis of pulmonary sarcoidosis was made and the patient was discharged on July 1, 1952, with instructions to report periodically to the chest clinic.

Two months later the patient was admitted to Cook County Hospital complaining of shortness of breath, inability to breathe while lying down, and swelling of his ankles. Physical examination at this time disclosed a grade III systolic murmur, moist pulmonary rales, hepatomegaly, and pitting edema of the ankles. The cardiac apex extended to the anterior axillary line, and the right cardiac border was percussed 2 cm. to the right of the sternal margin. The gamma globulin was 2.02 gm. per 100 ml. Chest films were essentially unchanged, and an electrocardiogram was interpreted as showing right axis deviation with prominent P

[1] From the Division of Laboratories, City of Chicago, Municipal Tuberculosis Sanitarium, and the Department of Pathology, Cook County Hospital, Chicago, Illinois.

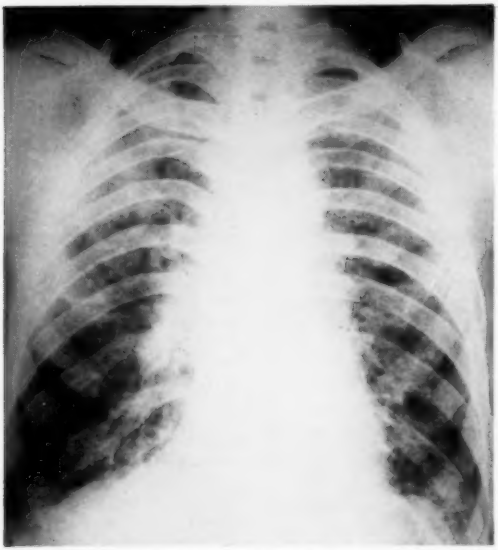


FIG. 1. Admission chest roentgenogram (June 6, 1951) showing a disseminated granular type of infiltration throughout both lung fields. The right hilum is prominent and there is blunting of both costophrenic sinuses.

waves. Corticotropin was given intravenously for three weeks with good results, and the patient was discharged to an outpatient cardiac clinic where he was maintained on digitalis and meralluride (Mercuhydrin®).

On August 21, 1956, the patient was readmitted to Cook County Hospital with marked shortness of breath. His blood pressure at this time was 95 systolic, 70 diastolic in mm. of mercury; pulse was 84 and irregular. There was a grade III blowing systolic murmur. The pulmonic second sound was accentuated. The liver was two fingerbreadths below the costal margin and slightly tender. A pretibial edema was present.

Urinary findings at this time were albumin 3-plus, hyaline and granular casts, and several erythrocytes. The erythrocyte count in the peripheral blood was 5,280,000 per cu. mm. The concentration of hemoglobin was determined as 87 per cent. The leukocyte count was 12,900 per cu. mm. with 29 per cent neutrophils, 9 per cent lymphocytes, and 62 per cent monocytes. Examination of the blood smear showed anisocytosis, 1-plus; toxic granules, 2-plus; smudge cells; and occasional polychromatophilia. Serologic tests for syphilis (Wasserman, Kahn) were doubtfully positive. The liver profile was consistent with liver dysfunction. Chest roentgenograms revealed a moderately enlarged, pear-shaped heart, prominence of pulmonary markings, obliteration of both costophrenic angles, and slight tracheal deviation to the left. Increased markings were present in the left apical region, and there appeared to be cyst formation. Bronchoscopic examination revealed no abnormalities, and sections of scalene lymph node taken for biopsy revealed only chronic lymphadenitis. Some improvement was noted following treatment with digitalis and diuretics, and while streptomycin, PAS, and cortisone seemed at first to produce beneficial results, the patient's condition gradually deteriorated. Chest films revealed pulmonary fibrosis, localized areas of emphysema, massive enlargement of hilar lymph nodes, large pulmonary arteries, and cardiac enlargement to the right and left. The pulmonary findings, however, never were in keeping with the degree of anasarca present. The patient became stuporous and died on October 19, 1956, approximately six years after the onset of his first symptoms and four and one-half years after tissue from the lung was taken for biopsy.

Necropsy

The *heart* weighed 500 gm. Some fibrinous adhesions were present between the epicardium of the right ventricle and the pericardial sac. The right ventricle formed the apex of the heart and the myocardium of the right ventricle was markedly thickened (figure 2). In addition to dilatation of the right atrium and ventricle, the tricuspid valve was significantly dilated. The auricular appendage on the right contained some thrombi. The aorta did not show any gross or microscopic evidence of syphilis.

The *liver* weighed 1,500 gm., and macroscopi-

FIG. 2. Photograph of the heart with the opened right auricle and ventricle. Note the dilatation of the chambers and the hypertrophy of the myocardial wall. There is thickening of the trabeculae carneae.

FIG. 3. The marked "honeycombing" of the lung is apparent. The bronchi and blood vessels are dilated.

cally there were large and small nodules rather sharply demarcated from the surrounding parenchyma. The surface made by cutting showed a severe passive congestion. Microscopically there was an uneven connective tissue proliferation with broad bands of cirrhotic tissue in the subcapsular zone. Within the parenchyma, however, there was a relatively normal architecture with extensive passive congestion which had led to fibrosis of the central zones. A characteristic change of cardiac cirrhosis was noted—the formation of central lobular ductules. It is believed that these ductules represent a transformation of liver cells rather than a proliferation of bile ducts. Many small granulomas (consisting of epithelioid cells) were noted in the central lobules about the bile ducts. The diagnosis of sarcoidosis was further substantiated by the presence of para-amyloid, a proteinic material giving a positive PAS reaction.

All of the *lymph nodes* examined had a similar granulomatous reaction with deposition of para-amyloid material.

The *pancreas* was not grossly unusual, but on microscopic examination a marked proliferation of small ductules was noted. These intercollated cells were scattered throughout the lobules—in some instances appearing between the acinar cells. That these cells interfered somewhat with circulation was attested to by the presence of small necrotic foci.

The pleural surface of either *lung* was somewhat thickened. Surfaces made by cutting showed a "honeycombing" of the parenchyma with several large cysts which had smooth glistening linings (figure 3). The bronchi were dilated and all of the lymph nodes were enlarged, firm, and pigmented blue-black. Microscopic sections of the lung revealed marked scarring with bronchial and bronchiolar dilatation, obstructive emphysema, deposition of para-amyloid, proliferation of

muscle fibers, and intimal changes in the pulmonary arteries with thrombi present in some branches.

Comment

The lungs are involved in sarcoidosis more than any other organ system. Gendel and his associates (2) reported 83 per cent pulmonary involvement in their series of twenty-four cases. A study of the sections from the lung biopsy specimen show four important changes: first, there is active sarcoid disease; second, bronchial involvement; third, bronchiolar proliferation; and fourth, distribution of para-amyloid throughout.

The earliest lesion of active sarcoidosis may be described as a discrete epithelioid granuloma consisting for the most part of epithelioid cells and giant cells, many of which are of the Langhans' type. Ricker and Clark (3) encountered a lesion which seemed to precede the development of sarcoid granulomas in some cases. This was present in lymph nodes and consisted of nonspecific proliferation of the littoral sinusoidal reticuloendothelium. They noted that the earliest granulomas regularly arose in juxtaposition to such a sinusoid, but attempts by serial sections to show that these granulomas developed from the proliferated reticuloendothelium failed. The fate of the sarcoid lesion cannot be consistently predicted. This lesion may remain practically unchanged for indefinite periods, it may resorb over

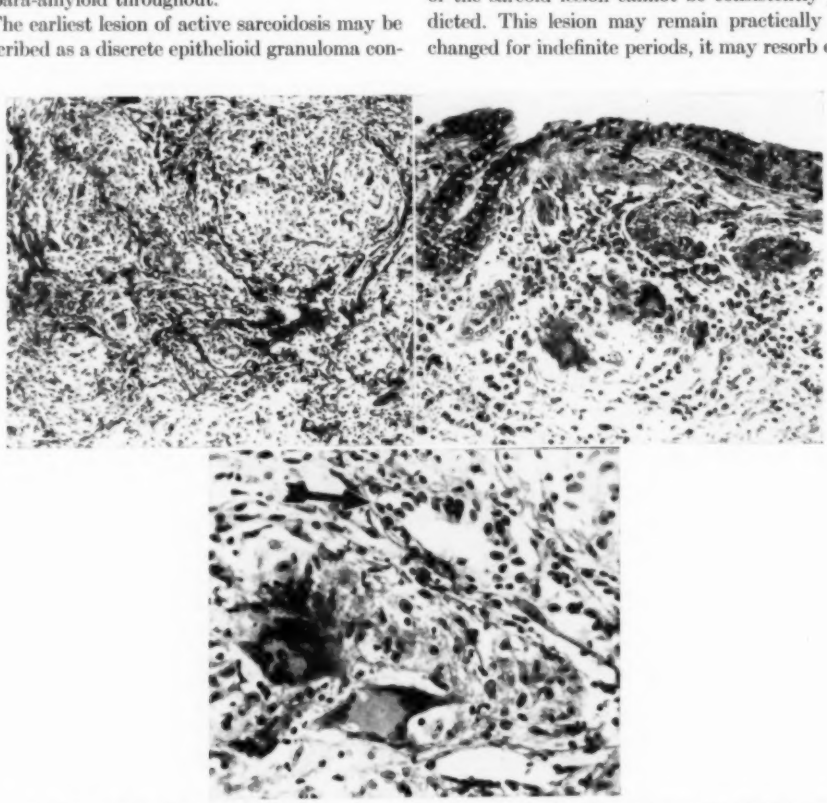


Fig. 4. (Upper left). This section of lung tissue shows complete replacement by sarcoid follicles composed of epithelioid cells and Langhans' type giant cells with strands of para-amyloid separating them. Biopsy taken June, 1952. (Hematoxylin and eosin stain, × 150)

Fig. 5 (Upper right). Photomicrograph of tissue taken for biopsy showing portion of bronchial wall. There is metaplasia of the lining epithelium, and a follicle with loosely arranged epithelioid cells and two multinucleated giant cells is noted. (Hematoxylin and eosin stain, × 120)

Fig. 6 (Bottom). Section of biopsy specimen showing bronchiolar proliferation (arrow) at the periphery of a follicle. The bronchiole is lined in part by columnar cells. (Hematoxylin and eosin stain, × 250)

a period of a few months, or a slow process of sclerosis may gradually transform the granuloma into a relatively acellular fibrous nodule (4). Repeated liver biopsies in a patient with jaundice reported by Wagoner and co-workers (5) showed a fairly consistent morphologic pattern of the disease over a two-year period.

The giant cells which are present may contain inclusion bodies (these are nonspecific for sarcoid). Longcope and Freiman (6) have described in detail three types of inclusions, namely, the asteroid or radio-inclusion, the lamellated or conchoidal bodies, and a poorly staining anisotrophic fragment representing a crystal or minute fragment of glass or silica. The second type of inclusion has been noted consistently in tuberculous cavity walls which have undergone "open-healing." Figure 4 is typical of the more involved areas of the lung with the loosely arranged epithelioid cells and giant cells and a small amount of para-amyloid separating the follicles. There is an absence of central caseation and peripheral "cuffing" with lymphocytes. The presence of reticulum is confirmed in these sections by specific staining procedures.

In several areas of the lung biopsy section, sarcoid follicles are noted beneath the mucosa of the bronchus (figure 5). The bronchial epithelium has undergone metaplastic change, but no ulceration is noted. Bronchial involvement in sarcoidosis has been reported in several cases and in most instances as morphologic evidence confirming the clinical and roentgenographic diagnosis of the disease.

The third prominent failure of the biopsy specimen is the marked bronchiolar proliferation (figure 6). The epithelium of these bronchioles ranges from cuboidal to tall columnar, and some of these ductal structures are dilated whereas others have a very narrow lumen.

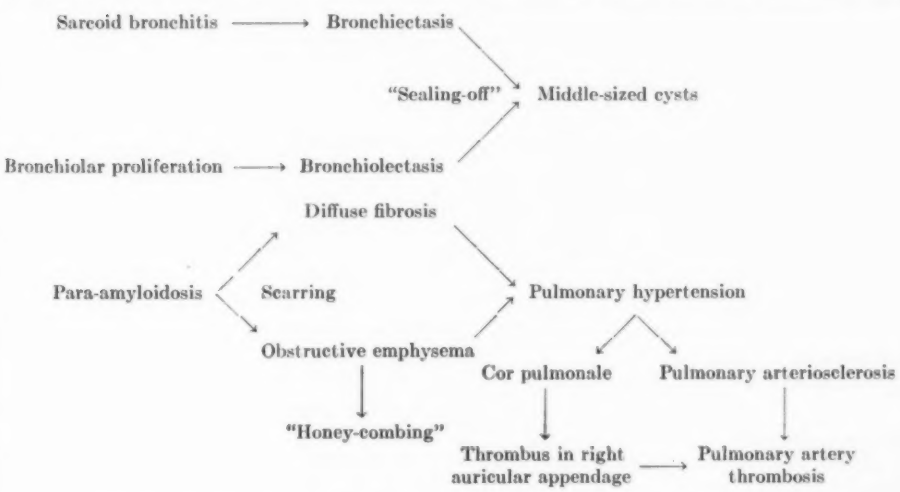
The last significant microscopic observation is the presence of para-amyloid material distributed throughout the sections (figure 4). This hyalin-like substance does not stain specifically for amyloid but does give a positive PAS reaction.

In examining the tissue sections from the necropsy, one sees the end stages of the morphologic changes observed in the biopsy sections. These changes can be illustrated graphically and may be seen in table 1.

As may be seen, the sarcoid follicles have undergone fibrosis with disappearance of the epithelioid cells and giant cells. There is some infiltration with lymphocytes. Most of the septal walls are increased in thickness due to fibrosis and there is obliteration of the vascular structures

TABLE 1

THE END STAGES OF THE MORPHOLOGIC CHANGES OBSERVED IN THE BIOPSY SECTIONS



(figures 7 and 8). The bronchi are tremendously dilated and show fibrosis of the submucosal layer along with alteration of the lining epithelium (figure 9). Likewise, the bronchioles are dilated and formation of new muscle fibers is noted as a characteristic feature of the acquired fibrosis (figure 10). This alteration of both the bronchi and bronchioles resulted in the formation of cysts of variable sizes. The para-amyloid which is noted in the biopsy sections likewise underwent scarring, with a resultant diffuse fibrosis of the interstitial tissue which, in turn, provoked an obstructive type of emphysema and a gross picture of "honeycombing" of the pulmonary parenchyma. As a sequel to these marked changes in the lungs, a hypertension of the lesser circulation developed with consequential right heart hypertrophy and dilatation (cor pulmonale). Associated with this were sclerotic changes in the pulmonary arteries. A thrombus was present in a branch of the pulmonary artery which probably represented an embolus from the right auricular appendage despite the fact that there was a severe degree of arteriosclerosis in the pulmonary vessels.

The patient was known to have had sarcoidosis for at least six years. In the necropsy sections, examination revealed the end stages of the mor-

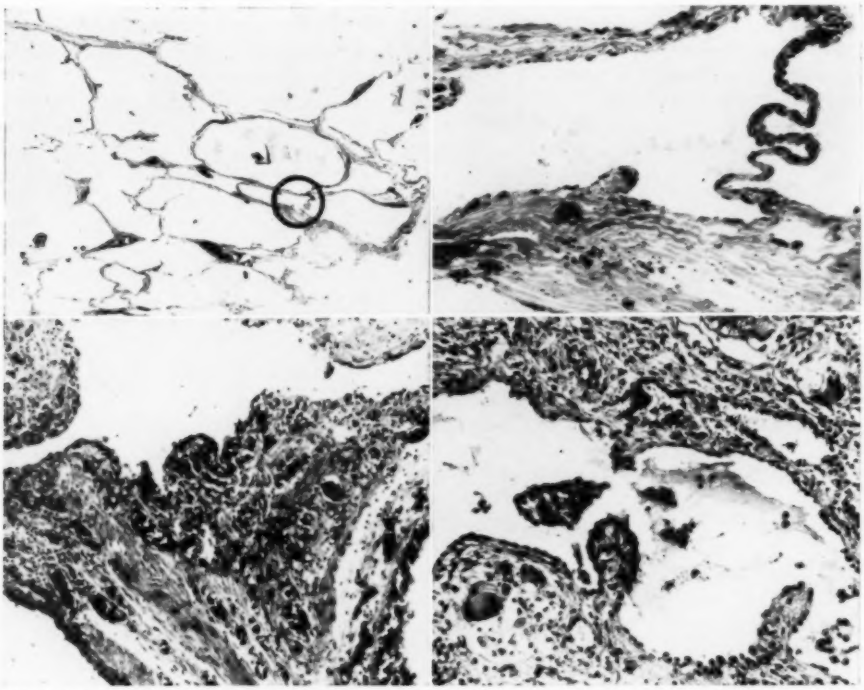


FIG. 7 (Top left). Low magnification of section from lung at autopsy showing marked dilatation of air spaces, thickening and scarring of intra-alveolar septae, and thickening of vessel walls. (Hematoxylin and eosin stain, × 12)

FIG. 8 (Top right). Higher magnification of circled area in figure 7. Note the marked para-amyloid and scarring of the septae. (Hematoxylin and eosin stain, × 150)

FIG. 9 (Lower left). Marked bronchiectasis as seen in the necropsy specimen. There is flattening of epithelium, infiltration with lymphocytes, scarring, and hypertrophy of muscle bundles. (Hematoxylin and eosin stain, × 100)

FIG. 10 (Lower right). Section of lung at autopsy showing marked dilatation of bronchioles which are lined for the most part by flattened epithelium. Small follicles are present. (Hematoxylin and eosin stain, × 100)

phologic alterations of the lung noted in the biopsy specimen. The epithelial proliferation accompanying this inflammatory process was revealed in the peculiar ductular sprouting in the lungs, liver, and pancreas. The elevation of the alkaline phosphatase value is believed to be a reflection of this ductular proliferation. The diffuse fibrosis of the pulmonary parenchyma led to marked emphysematous change and right heart enlargement with a terminal clinical picture of myocardial failure.

Summary

The evolution of pulmonary sarcoidosis from the florid stage to the terminal stage was revealed in sections of lung tissue taken for biopsy and in necropsy material obtained four and one-half years later. The salient microscopic changes were: active sarcoidosis, bronchial involvement, bronchiolar proliferation, and para-amyloidosis. The end stages as noted in the tissues from necropsy included bronchiectasis and bronchiolectasis, diffuse fibrosis of the lungs with emphysema and "honeycombing," and marked scarring of the para-amyloid.

Sumario

Evolución de la Sarcoidosis Pulmonar

La evolución de la sarcoidosis pulmonar desde el período florido hasta el terminal quedó revelada en cortes de tejido pulmonar extirpado para la biopsia y en material autópsico obtenido cuatro años y medio después. Las sobresalientes alteraciones microscópicas fueron: sarcoidosis activa, invasión bronquial, proliferación bronquiolar y paraamiloidosis. Las fases terminales, según se notaron en los tejidos derivados de la autopsia, comprendieron bronquiectasia y bronquiolectasia, fibrosis difusa de los pulmones con enfisema y "apanalamiento" y acentuada cicatrización en la porción afectada por la paraamiloidosis.

Resume

Evolution de la sarcoïdose pulmonaire

L'évolution de la sarcoïdose pulmonaire du stade floride au stade terminal a été révélée dans des coupes de tissus pulmonaires prélevées par biopsie et au cours de l'autopsie pratiquée quatre ans et demi plus tard. Les signes anatomo-pathologiques saillants étaient les suivants: sarcoïdose active, lésion bronchique, prolifération bronchiolaire et para-amyloïdose. Au stade terminal les lésions observées dans les pièces d'autopsie comprenaient la bronchectasie et la bronchiolectasie, la sclérose diffuse des poumons avec emphysème et les lésions cicatricielles marquées en nid d'abeilles de la para-amyloïdose.

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SILICOSIS AND AVIAN TUBERCULOSIS[1]

RICHARD P. JAHN

(Received for publication February 27, 1959)

Introduction

It has been generally accepted that human infection with avian tubercle bacilli is extremely rare. Reports presenting credible evidence in proof have been few. Considering the widespread prevalence of infection in fowl (2 to 6 per cent) and swine (1–3), it is obvious that opportunity for infection among humans is considerable. This fact alone indicates that humans must possess a high degree of immunity to avian tubercle bacilli.

Under certain conditions, however, the resistance of a host can be so altered that progressive infection with an otherwise avirulent strain of tubercle bacilli can be induced. Vorwald (4), Gardner (5), and Policard (6) infected guinea pigs exposed to silica. That a similar situation might obtain in humans is suggested by Karlson's report (7) of avian tuberculosis in an individual with silicosis.

Case Report

A 58-year-old Negro male foundry worker for twenty-five years was admitted to Muirdale Sanatorium in August, 1949, as a result of a sputum smear positive for tubercle bacilli, obtained at the time of his last yearly foundry-worker examination. His only complaints were a slight nonproductive cough present for many years and exertional dyspnea noted for about one year.

Fig. 1. Maximal progression of the disease process as it appeared before death.

On physical examination, the patient appeared to be well nourished, well developed, and in no acute distress. There was mild clubbing of the fingers. Blood pressure was 148 systolic, 76 diastolic in mm. of mercury. Pertinent chest find-

[1] From Muirdale Sanatorium, Milwaukee 13; and Marquette University School of Medicine, Milwaukee 3, Wisconsin.

FIG. 2 (Left). Earliest evidence of nodulation.
FIG. 3 (Center). Nodulation is well developed with hilar involvement but without clear-cut evidence of conglomeration.
FIG. 4 (Right). Conglomeration is now present, indicating that infection has occurred between 1945 and 1949.

TABLE 1

COMPLETE BACTERIOLOGIC FINDINGS FROM 1948–1956

Date	Specimen	Direct or Homogeneous	Culture	Guinea Pig	Remarks
8–29–49	48-hour sputum	Positive	Acid-fast bacilli, 9–15–49	—	—
9–28–49	48-hour sputum	Negative	Acid-fast bacilli, 10–19–49	—	—
10–26–49	24-hour sputum	Negative	Negative, 12–21–49	—	—
12–21–49	3 pooled 48-hour sputum specimens	Negative	Negative, 2–24–50	—	—
3–1–50	3 pooled 48-hour sputum specimens	Negative	Acid-fast bacilli, 3–31–50	Negative	Guinea pig inoculation from culture, 3–31–50; sacrificed, 5–23–50.
3–6–50	5 pooled gastric washings	—	Acid-fast bacilli, 4–15–50	—	—
5–5–50	3 pooled gastric washings	—	Acid-fast bacilli, 6–8–50 chromogenic	—	—
7–17–50	1 gastric washing	—	Acid-fast bacilli, 8–9–50	Negative	Guinea pig inoculation with direct pooled specimen, 7–20–50; sacrificed, 8–10–50.
7–18–50	1 gastric washing	—	Acid-fast bacilli, 8–9–50		
7–19–50	1 gastric washing	—	Acid-fast bacilli, 8–9–50		
9–18–50	3 pooled gastric washings	—	Acid-fast bacilli, 10–11–50	—	—
9–20–50	3 pooled 48-hour sputum specimens	Negative	Acid-fast bacilli, 10–26–50	—	—
1–24–51	48-hour sputum	Positive	Acid-fast bacilli, 2–23–51	Negative	Guinea pig inoculation from culture, 2–23–51; sacrificed, 3–27–51.
4–26–51	48-hour sputum	Positive	—	—	—
7–5–51	48-hour sputum	—	Acid-fast bacilli, 8–3–51	Negative	Guinea pig inoculation from culture, 8–3–51; sacrificed, 9–7–51.
7–9–51	48-hour sputum	—	Acid-fast bacilli, 8–3–51	Negative	Guinea pig inoculation, 8–3–51; sacrificed, 8–27–51.
11–12–51	3 pooled 48-hour sputum specimens	Negative	Acid-fast bacilli, 12–13–51	—	—
7–8–52	48-hour sputum	Positive	Contaminated, 8–5–52	—	—
7–29–52	48 hour sputum	Positive	Acid-fast bacilli, 9–25–52	—	—
9–22–52	3 pooled 48-hour sputum specimens	Negative	Acid-fast bacilli, 11–11–52	—	—
11–10–52	48-hour sputum	Acid-fast bacilli	—	—	—

11–10–52	3 pooled 48-hour sputum specimens	Acid-fast bacilli	Acid-fast bacilli, 12–5–52	—	—
2–16–53	3 pooled 48-hour sputum specimens	—	—	Negative	Untreated specimen, guinea pig inoculation, 2–24–53; sacrificed, 4–13–53.
2–24–53	3 pooled 48-hour sputum specimens	—	—	Negative	Treated specimen, guinea pig inoculation, 2–24–53; sacrificed, 4–13–53.
10–12–53	48-hour sputum	Negative	Contaminated, 10–22–53	—	—
10–15–53	3 pooled gastric washings	—	Acid-fast bacilli, 11–5–53	Negative	Guinea pig inoculation, 11–5–53; sacrificed 12–31–53. Caseous abscess abdominal wall; acid-fast organisms in smear; no involvement of internal organs.
10–29–53	48-hour sputum	Negative	Acid-fast bacilli, 11–12–53	—	—
2–8–54	48-hour sputum	—	Acid-fast bacilli, 3–4–54	—	—
11–15–54	48-hour sputum	Positive	Acid-fast bacilli, 12–22–54	—	Guinea pig inoculation, 12–23–54; Report lost.
11–15–54	3 gastric washings	—	Acid-fast bacilli, 12–10–54	—	—
3–21–55	3 gastric washings	—	Acid-fast bacilli, 4–12–55; atypical morphology	Negative	Guinea pig inoculation, 4–12–55; sacrificed, 5–20–55. Abscess at site of inoculation. No TB in pig.
4–7–55	48-hour sputum	—	Contaminated, 5–5–55	—	—
4–8–55	Urine	—	Negative, 6–3–55	—	—
5–13–55	48-hour sputum	—	Atypical colony acid-fast bacilli; atypical morphology 6–3–55	Negative	Guinea pig inoculation, 6–3–55; sacrificed 7–8–55.
8–7–55	3 gastric washings	—	Smooth colonies acid-fast bacilli; atypical morphology, 8–25–55	?	Guinea pig inoculation, 8–25–55; sacrificed 9–19–55. Small subcutaneous abscess at site of inoculation. Internal organs negative by gross and microscopic examination. Subsequent guinea pig inoculated from same culture showed no TB.
10–10–55	3 gastric washings*	—	Acid-fast bacilli,* 10–27–55	Negative	10–27–55, atypical morphology and smooth colonies.
12–27–55	3 gastric washings*	—	Acid-fast bacilli,* 1–12–56, photochrome	Negative	Atypical morphology; smooth colonies. Above was exposed to light 1–12–56; became chromogenic 1–19–56.
4–6–56	48-hour sputum	—	Acid-fast bacilli, 5–1–56	—	Atypical morphology; smooth colonies.

* These are the specimens which were studied by Dr. A. G. Karlson.

ings were an impaired percussion note and fine, moist rales over the upper third of the chest bilaterally, more pronounced on the left. A chest roentgenogram (figure 1) showed nodulation throughout both lungs with conglomeration in the upper half bilaterally. Review of earlier roentgenograms (figures 2, 3, and 4) showed the onset of nodulation in 1938, superimposed infection as shown by conglomeration appearing between 1945 and 1949.

Laboratory data included: erythrocyte count, 4.73; leukocyte count, 6.35 with a normal differential count. Serologic tests for syphilis were: Eagle and Wasserman, positive; Kline, 4-plus. The avian tuberculin test, 1:10,000, was positive, 15 × 20 mm. in size; the reaction to PPD 0.00002 was negative; to 0.005, positive, 30 × 30 mm. in size.

Initial streptomycin therapy, 1.3 gm. daily in divided doses, was discontinued after forty-two days because of vertigo. By November, 1950, repeated failure to obtain bacilli which were pathogenic for guinea pigs resulted in the patient's discharge. In June, 1951, a sputum smear positive for tubercle bacilli at the time of a re-employment examination resulted in readmission. During the seven-year period of hospitalization, therapy consisted of streptomycin and PAS with the addition of isoniazid in 1952. Interruption in treatment occurred at irregular intervals due to recurrent dermatitis and vertigo.

In October, 1955, pyrazinamide was administered for a period of one month. In November, 1956, an episode of congestive heart failure was successfully treated with digitalis, diuretics, salt restriction, and oxygen. Recurrence of congestive heart failure in February, 1957, was intractable, and death occurred on February 14, 1957. No autopsy was obtained.

Complete bacteriologic findings from 1948 are shown in table 1. The bacteriologic investigation was done by Dr. A. G. Karlson on cultures of October 27, 1955, and January 12, 1956 (gastric aspirations).

On the original medium both cultures had smooth, buff-colored colonies resembling those of avian tubercle bacilli. The growth formed a uniformly turbid suspension when suspended in water; this is typical of avian tubercle bacilli. (Human and bovine types will not form uniform suspensions in water, but are coarsely granular.) Subcultures on Proskauer and Beck liquid medium formed a fine, turbid growth. (Human and bovine strains form a pellicle and a granular sediment.) Subcultures on egg-yolk agar and on Löwenstein medium grew in seven to ten days to form smooth moist colonies resembling avian tubercle bacilli. Both cultures were neutral-red positive, catalase positive, and failed to form cords.

Susceptibility tests: On egg-yolk agar medium both cultures were resistant to more than 10γ of isoniazid and to more than 10γ of PAS per ml. of medium. Both were resistant to 10 but not to 50γ of streptomycin. These results are typical of avian tubercle bacilli.

Agglutination reactions: An antigen was prepared from the second culture (12-356) by suspending the microorganisms in 0.85 per cent NaCl solution containing 0.5 per cent phenol. Similar antigens were prepared from four cultures of avian tubercle bacilli which had been isolated from tuberculous chickens. The antigen prepared from the culture was agglutinated by serum from two tuberculous chickens but not by serum from a normal chicken or by normal human serum. It was also agglutinated by serum from the patient (obtained December 30, 1955). The patient's serum agglutinated three of the four antigens prepared from known cultures of avian tubercle bacilli.

Animal pathogenicity tests: Suspensions were made in sterile water from each of the original cultures at a density of about tube No. 0.5 of McFarland's nephelometer or about 300 parts per million of silica gel standard. Each culture was used to infect two guinea pigs, two rabbits, and two chickens. (The chickens were tested previously with avian tuberculin and had failed to react.) The rabbits and chickens were each given 1.0 ml. of the suspension intravenously. The guinea pigs were infected subcutaneously with a similar amount.

The rabbits infected with the first culture died in eighteen and nineteen days, respectively. Each had enlargement and infarction of the spleen, smears of which had innumerable acid-fast bacilli. The chickens died in twenty-six and thirty-one days, respectively, each having marked enlargement of the spleen and liver. Microscopy of these organs revealed many acid-fast bacilli. Histopathologic examination revealed a widespread exudative type of lesion with many acid-fast bacteria, which is typical of experimentally induced avian tuberculosis in rabbits and chickens. The 2 guinea pigs were tested with avian and mammalian tuberculin in sixty days (0.1 ml. of 1:100 OT). A 4-plus reaction for avian tuberculin and a 2-plus reaction for the mammalian were recorded. The guinea pigs were killed ninety days after inoculation and were found to have no tuberculous lesions in the internal organs.

The two rabbits infected with the second culture died in twenty-one days and the chickens died in twenty-nine and thirty-five days, respectively. The gross and microscopic appearance was the same as described above. The guinea pigs were tested with avian and mammalian tuberculin in sixty days. One animal had a 4-plus reaction to both tuberculins and the other had a 4-plus reaction to avian and a 1-plus reaction to mammalian tuberculin. These two guinea pigs were killed in ninety days. They had no tuberculous lesions of the internal organs.

Comment

Increasing incidence of authenticated human infection by atypical variants of Mycobacterium has become widespread. Attention is again being focused in this connection upon the avian bacillus. Review of previous reports reveals that controversy has revolved around two points: positive identification of the bacillus; and proof that the avian bacillus has been the causative organism in the disease at hand.

Feldman (8) demanded these proofs: That the bacillus isolated from human source should show cultural, antigenetic, and pathogenetic patterns of the avian bacillus; and that there should be isolation of the proved avian bacillus from tissues of laboratory animals inoculated with material from the human source.

Rich (9) posed these criteria: That there must be failure to cause tuberculosis in guinea pigs inoculated with a small subcutaneous dose; that disease must be caused in a rabbit inoculated with a small dose; that disease must be caused in tuberculin-negative fowl inoculated with a small dose; and, if possible, that there should be other bacillary characteristics, including cultural agglutination response and production of avian tuberculin.

Rich also emphasized the inadequacy of reports, particularly regarding numbers and types of animals used, dosage and route of inoculation, and extent, type, bacillary content, and histology of the lesions found in the laboratory animals. In essence, these demands insist upon the fulfillment of Koch's postulates. Thus qualified, it is apparent that avian infection in normal humans is a rare occurrence.

The question remains, however, of infection with normally avirulent bacilli under circumstances in which it is reasonable that there may be interference with normal resistance patterns. There is evidence to suggest that silicosis is one of these conditions (4–6). Karlson (7) has reported one such case.

In the present case, the industrial exposure, the roentgenographic findings, the clinical picture, and the course of the disease were characteristic of silicosis. There were repeated isolations of acid-fast bacilli, atypical in character and negative for the guinea pig, which animal studies conclusively demonstrated as avian tubercle bacilli.

Summary

In a case followed for twenty-one years, the development of silicosis and a superimposed infection was observed. In addition to a positive avian tuberculin test, atypical acid-fast bacteria were recovered repeatedly and constantly over a ten-year period. These bacilli with cultural qualities, agglutination studies, and drug-resistant patterns typical of avian tubercle bacilli showed consistent inability to infect a guinea pig and ability to infect fowl.

Sumario

Silicosis y Tuberculosis Aviaria

En un caso mantenido bajo observación veintiún años, se observó la aparición de silicosis y de una infección sobrepuesta. Además de una prueba positiva a la tuberculina aviaria, se obtuvieron repetida y constantemente bacterias ácidorreistentes atípicas durante un período de diez años. Estos bacilos con propiedades culturales, estudios de aglutinación y patrones farmacorresistentes típicos de los bacilos tuberculosos aviaria demostraban constante incapacidad para infectar a un cobayo y capacidad para infectar las aves de corral.

Resume

Silicose et tuberculose aviaire

Dans un cas suivi pendant vingt-et-un ans le développement de la silicose et d'une infection secondaire a été observé. Outre un test à la tuberculine aviaire positif, des bactéries atypiques acido-résistantes ont été isolées de façon répétée pendant une période de dix années. Ces bacilles avec qualités des cultures, etudiés de l'agglutination et les modalités de résistance aux drogues caractéristiques de la bacille tubercule aviaire ont démontré d'une part une inaptitude constante à l'infection du cobaye et d'autre part la capacité d'infecter la volaille.

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Notes

EFFECTIVENESS OF ANTITUBERCULOUS TREATMENTS TESTED BY DIRECT CULTURE OF THE BACILLI IN THE PATIENT'S BLOOD

Some ten years ago, there was reported from this laboratory[1] a method of assessing the effectiveness of antibacillary medications based on the principle of ascertaining whether the bacilli of the patient can actually multiply *in vitro* in blood obtained from the patient after a given drug has been administered. Since then considerably more studies have been made with this method. It has been used especially in patients receiving multiple-drug therapy, and in these circumstances appears to be particularly useful.

In essence, the principle of the method is based on the long-established observation that *M. tuberculosis* readily multiplies in pure heparinized human blood in slide cultures. Accordingly, blood from an individual patient with known doses of antibacillary drugs added would presumably be the most appropriate medium for determining the drug resistance of that patient's strain of *M. tuberculosis*. With the method employed in this laboratory, however, there is no need to add drugs directly to the blood. The procedure used is simply to take a sample of the blood from the patient under treatment. Then, if under the conditions of the test it is found that the patient's own strain of tubercle bacilli does multiply in the blood sample, the obvious conclusion is that the treatment in progress would not be expected to bring about any positive action.

A blood-sample of approximately 10 ml. is taken before the start of treatment and a further sample of 10 ml. at the time after the drugs have been administered when it is assumed that the drug concentration is at its highest (usually two hours after administration). The blood is poured into small sealed vials containing a trace of dry heparin. In the laboratory, 5 ml. are placed into an ordinary 16-mm. test tube, while the remaining 5 ml. are used to form 1:4 and 1:16 dilutions in Dubos medium. Following this, 5 ml. of each dilution are then poured into individual test tubes.

The sputum of the patient is then examined under a microscope to ensure that it contains a sufficient amount of tubercle bacilli. The sputum is smeared on the lower end of twelve narrow slides (normal slides being cut in half, longitudinally, for the purpose). Once the smear is dry, innocuous microbic flora will be destroyed by immersing the slides during seven minutes in a 6 per cent solution of sulfuric acid, and then rinsing them successively in two Petri boxes filled with sterile distilled water. The slides are then immersed, two by two and back to back (separated by a piece of glass), smear downwards, in the test tubes containing pure blood and diluted blood. The tubes are then placed in an incubator.

After a period of seven days, one of the two slides is removed from each of the test tubes and washed in a beaker. Carbol fuchsin dye is then used. The other slides remain in the incubator until the fourteenth day.

Microscopic observation is carried out with low-power magnification, slides being coated with immersion oil. The extent of the bacillary multiplication in the blood samples taken before and after the drugs were administered is then compared. When no multiplication of bacilli is evident in the sample of blood taken before the administration of drugs, the results will have to be ascertained from the second slide on the fourteenth day only.

The results are recorded as: *growth equal to control; partial growth; no growth.*

The use of sputum avoids any arbitrary choice of the number of bacilli and has the advantage of being a precise representation of the pathologic product to be sterilized. When no sputum is available or when no tubercle bacilli can be seen under the microscope, recourse must be had to a culture obtained in Löwenstein medium; this being subsequently transferred to Tween® 80 Dubos medium.

Once the culture is four to six days old, it is placed in a centrifugal machine and rotated for one to three minutes at 1,500 r.p.m.. After this operation, 0.5 ml. of the liquid from the surface is removed and mixed with white of egg in equal proportions. Five-hundredths of a milliliter of the mixture is smeared on each slide.

This method makes it possible to obtain information with a single test on the antimicrobial potency of the blood of patients receiving the multiple-drug regimens that are used routinely in this institution. For the present purposes, however, it is easier to compare the results obtained during single-drug therapy with the resistance of the bacilli to the drug.

A definite agreement between the results and the resistance *in vitro* of the bacilli is shown in table 1. Strains of tubercle bacilli which are

[1] BERNARD, E., AND KREIS, B.: Rev. tuberc., Paris, 1949, *13*, 737.

TABLE 1

EXTENT OF GROWTH OF A PATIENT'S OWN TUBERCLE BACILLI IN HIS OWN BLOOD

Bacteriologic Resistance	Number of Patients	Pure Blood			Blood Diluted 1:4			Blood Diluted 1:16		
		Culture Abundant	Culture Partial	Culture Nil	Culture Abundant	Culture Partial	Culture Nil	Culture Abundant	Culture Partial	Culture Nil
Isoniazid, 0.15 gm. orally two hours before taking of blood sample										
Susceptible	38	1	5	32	2	8	28	1	14	23
$0.1 < R < 1$	6	2	4	0	5	1	0	5	1	0
$1 < R < 5$	5	3	2	0	4	1	0	4	1	0
$5 < R < 50$	2	1	1	0	2	0	0	2	0	0
$R > 50$	9	4	5	0	7	2	0	7	2	0
Streptomycin, 1 gm. intramuscularly two hours before taking of blood sample										
Susceptible	25	1	0	24	1	7	17	2	9	14
$2 < R < 10$	8	0	5	3	3	5	0	3	5	0
$10 < R < 100$	7	2	2	3	2	4	1	4	2	1
$R > 100$	7	4	3	0	6	1	0	6	1	0

susceptible to a given drug do not multiply in the blood samples taken during treatment. In fact, growth equal to control appears only once out of 38 separate observations in the case of isoniazid and once out of 25 tests in the case of streptomycin. The method further detects some differences between the various drug-susceptible strains. Most of the strains (23 of 38 for isoniazid; 14 of 25 for streptomycin) are totally inhibited by the 1:16 dilution of the blood; some are inhibited only by the 1:4 dilution of the blood (5 of 38 for isoniazid; 3 of 25 for streptomycin). A few strains are totally inhibited only by pure blood (4 for isoniazid; 7 for streptomycin). Five strains of 38 are only partially inhibited even by pure blood under treatment by isoniazid. These findings seem to mean that the same amount of the drug taken orally produces various concentrations in the blood in different patients, or that the bacilli said to be susceptible are not always of the same degree of susceptibility.

On the other hand, the majority of strains with reduced susceptibility to the drugs are capable of growing in the patient's blood under treatment. It is obvious, however, that the degree of drug resistance is not the only factor involved. For example, growth was obtained in the case of any degree of resistance to isoniazid. Thus, it is proper to conclude that there is no one "clinically significant" degree of resistance. The smallest enhancement of resistance may be significant and the end-result depends upon the concentration of active drug in the blood.

The amount of growth of a strain resistant to a drug is not always equal in the drug-containing blood to the growth which is seen in blood withdrawn before treatment with the drug in question. This discrepancy means that these particular strains contain only a small number of bacilli resistant to that particular drug. In such cases, treatment with the drug may eventually be of some value despite the drug resistance *in vitro*.

It is believed that this technique deserves to be more widely known. For, it has become a fairly general practice today in determining drug susceptibility for the bacteriologist to determine the minimal concentration of a given drug which effectively prevents the growth *in vitro* of the bacilli concerned. The clinician, in turn, compares these figures with those representing the degree of concentration of each drug which he assumes exists in the plasma of his patients. He considers the strain to be "clinically resistant" when his assumed concentration of drug is lower than that required for the inhibition of the bacilli *in vitro*.

There are two major objections that can be made to this procedure: the susceptibility *in vitro* of a given strain of tubercle bacilli is only of relative value; and there is a considerable person-to-person variation in the concentration of a drug attained in the blood on a given dosage.

With respect to the merely relative value of the *in vitro* result, it is known that the minimal inhibitory concentration of a drug can vary to an appreciable extent according to the number of bacilli involved in the test, the type of culture medium, the day when the test is recorded, and the number of colonies taken as significant. Moreover, the test results refer only to the susceptibility of the bacilli to each individual medicament

taken separately. Usually, however, patients are treated with two or three drugs simultaneously. Consequently, the over-all susceptibility of the tubercle bacilli cannot be deduced by more or less summing up their susceptibilities to each of the single drugs administered; possible antagonisms and synergy have to be considered. To be sure, experiments of this kind could be carried out *in vitro*, but they would be extremely tedious. With three drugs tested in three different concentrations, there would be no less than twenty-seven combinations that would have to be examined.

With respect to the individual variation in the pharmacologic distribution of drugs, it is well recognized that the concentrations of the different drugs obtained in the blood vary from person to person. This was emphasized in a previous report from this laboratory ten years ago in the case of streptomycin.[1] It was noted that with an injection of 0.50 gm. of streptomycin, the concentration of drug in the blood could vary as much as from 7.5 γ per ml. to 19.5 γ per ml., as determined by microbiologic testing. Middlebrook and his associates have recently stressed the extent of the individual variation of isoniazid concentrations attained and have advocated a systematic dosage to account for this variation. Moreover, not only is there a considerable person-to-person variation in drug absorption and distribution, but the concentrations in the blood also vary in the same patient according to conditions of treatment. Quite obviously, the concentrations of drug in the blood will be different when in one case three separate doses of 100 mg. of isoniazid are prescribed daily and, in another, one single dose of 300 mg. is administered each day.

The difficulty in estimating the correct amount of drug in the blood is far greater when a patient is being treated with several drugs. Here, two other factors have to be allowed, i.e., all of the drugs are not usually administered at exactly the same time of the day, and the presence of one drug may cause the concentration of one or the other to vary. In point of fact, it is believed by some observers that the presence of PAS in the blood affects the concentration of isoniazid.

In brief, present methods and procedures do not give the clinician the accurate information he requires as to the susceptibility of the bacilli to the combined effect of several drugs. Furthermore, the clinician receives no precise indication of concentrations of drug that are attained in the blood.

To be sure, these conventional methods are presumably valid in the case of very large-scale variations in microbial susceptibility, i.e., in which it is considered certain that the drug does not affect the bacilli. Nevertheless, the usual methods do not meet the present desire of the clinician, which is to know which drug he can usefully combine with which other drug against moderately resistant bacilli. It is believed that much of this criticism is not applicable to the present method which has the additional value of being technically very simple, most economical, and yielding much more rapid results.

Absence of any inhibition of the bacilli during treatment may be due to any of several reasons; most often the cause is a *drop in the susceptibility of the bacillus* to drugs but, in other cases, the *concentration of antimicrobially active drug in the body fluids* may be insufficient. This can be ascribed to either a too rapid elimination or destruction of the medicaments or insufficient dosages and possible administration of a drug at the wrong time of the day, e.g., too soon after eating. Apart from these causes, the reason may also be the existence of antagonistic substances in the plasma, irrespective of whether such substances are of endogenous origin or absorbed by the patient. On the other hand, it must be remembered that the actual bacteriostatic power of the blood-serum, in preventing the multiplication of resistant bacilli, can act in conjunction with that of the drugs. One of the most important advantages of the present method is that it takes into account the above factors. As seen below, the method makes it possible to define these various causes; however, what is important for the clinician is the aggregate and ultimate result.

As will be noted, the present method does not produce any numerical values for either the quantity of drug present in the blood or the drug susceptibility of the microorganisms. The aim of the procedure is to test the action of the humoral medium of the patient against his own bacilli in order to assess the possible effectiveness of the treatment. If the bacilli continue to multiply, it seems clear that the treatment cannot be effective. By contrast, the fact that inhibition occurs does not mean that the treatment must be effective.

TABLE 2

STUDY OF AMOUNT OF ACTIVE ANTIMICROBIAL DRUG IN BLOOD

Effectiveness of Cycloserine Tested on H37Rv-Susceptible Bacilli in the Blood Collected from Several Patients (Sample of Patient's Blood Being Taken Two Hours after Having Absorbed 0.25 Gm. of Cycloserine)

Number of Patients	Culture		
	Abundant	Partial	Nil
41	10	16	15

Concentration of drugs in the lesions may be less than in the blood. All that can be established by this method is whether the treatment *can* be effective.

The method also makes it possible to study the various causes involved in the growth of the bacilli. It will be found easy to determine the bacteriostatic power of the patient's blood (comparison of a slide in the patient's blood without drug with another placed in standard blood) or the bactericidal power of the treatment prescribed (by placing a slide first in the blood with the drug and some time after in an ordinary culture medium).[2] It is also possible to study the effective action of treatment by injections at two- or three-day intervals[3,4] or to obtain information on the inhibiting or growth-stimulating action of various food products or therapeutic substances (vitamins, cortisone, et cetera) on the development of the bacilli.

A study on the *blood antimicrobial drug concentration*, in this case cycloserine, is shown in table 2. The same procedure is used once again but, instead of bacilli from patients, all that is required is a stock strain of drug-susceptible bacilli (H37Rv). When no inhibition of the culture takes place, this means that the quantity of antimicrobial drug in the blood is insufficient. This occurred in 10 of 41 patients. In the case of the other 31 patients, however, the substance had a marked effect, although the bacillary inhibition was only complete in 15 cases. It may be seen how easy it is to check a particular treatment regimen by such a use of the present method. It is also possible with the same method to determine the effectiveness of serum concentration of a drug in a given patient at various time intervals after the administration of different doses of the drug.[5]

In summary: A method is described whereby tubercle bacilli from a patient are grown *in vitro* in the patient's blood obtained before and after the administration of drugs. A comparison of these data with those obtained by conventional drug-susceptibility tests revealed that the patient's own bacilli were virtually always inhibited by his own blood withdrawn in the beginning of treatment with streptomycin or isoniazid. At times, the patient's own bacilli were not inhibited during the treatment with a weaker drug such as cycloserine. As for the patient-strains that have lost some drug susceptibility, some which appear to be highly resistant to conventional testing give only partial or no growth in the host's own blood. This is principally a result of the fact that some strains of tubercle bacilli are only partially resistant to a particular drug, and that the blood concentrations of a drug may vary considerably in different patients. It is believed that the method described can give more accurate information to the clinician than the conventional tests for drug susceptibility *in vitro*, especially in patients receiving multiple-drug therapy.

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[5] BERNARD, E., AND KREIS, B.: Ann. Inst. Pasteur, 1951, *80*, 87.

CYCLOSERINE-ISONIAZID IN THE AMBULATORY TREATMENT OF ACTIVE TUBERCULOSIS AFTER FAILURE OF PREVIOUS CHEMOTHERAPY

Since 1955, an increasing number of reports have appeared on the results of cycloserine in the treatment of pulmonary tuberculosis. Because of early emphasis on toxicity, cycloserine has so far been administered only to hospitalized patients.

In an attempt to determine whether cycloserine could have a place in the ambulatory treatment of tuberculosis, the medical staff of the Bureau of Tuberculosis of the New York City Health Department decided in the spring of 1957 to start a small selected group of patients on this drug. This study was primarily intended as a study of toxicity and, secondarily, as a therapeutic trial. No reports on patients newly started on cycloserine in outpatient departments are presently available in the literature. On the contrary, warnings urging caution in the use of cycloserine in ambulatory patients have been repeatedly expressed, pointing to the danger of neurotoxic reactions.[1-3] However, other studies,[4, 5] as well as the writer's experience,[6] indicated relatively low toxicity with reduced cycloserine dosage. It was hoped that with small daily amounts of cycloserine in divided doses it would be possible to reduce toxic effects to a minimum, to obtain some data on the practicability of the ambulatory use of cycloserine and, at the same time, to offer a selected group of patients the possible benefit of an additional antituberculous agent.

The 56 patients selected for this study all had active far advanced tuberculosis of long standing, the duration of disease ranging from two to twenty-nine years (average, seven years). All patients had previously been unsuccessfully treated with long courses of the three major antituberculous drugs (streptomycin, isoniazid, and para-aminosalicylic acid) in hospitals as well as on an ambulatory basis; many had also been treated with collapse therapy or resectional surgery. The sputum had remained positive for tubercle bacilli in all. At the time when cycloserine was started, the patients were on an ambulatory regimen of chemotherapy which included isoniazid.

All of the patients consistently refused hospitalization for a variety of personal reasons, but were under regular clinical supervision and did not represent a significant public health menace. It was believed that for these patients the use of an additional antituberculous drug was medically indicated and that, in view of the seriousness of their disease, it was justifiable to use a drug with a certain toxic potential.

In order to minimize the toxic effects of cycloserine, the following categories of patients were excluded from the study: patients over the age of 65; severe alcoholics; patients with a history of neurologic or mental disorders; and very unreliable, unstable, or uncooperative patients.

Of the 56 patients, 42 were males and 14, females. The ages ranged from twenty-three to sixty-three years, averaging forty-six years; 23 patients were fifty years and older. The ethnic and socioeconomic background was varied and reflected the general clinic population of New York City, the patients having been selected from all of the district chest clinics of the Health Department.

Pretreatment observations included history taking, complete physical examination, chest roentgenogram, and collection of sputum specimens for culture of tubercle bacilli. There were no facilities for hematologic examination, or for tests of renal and hepatic function. After cycloserine was started, the patients were seen in the clinic at weekly intervals for the first two months and at monthly intervals thereafter. Sputum specimens were collected at each monthly clinic visit. Drug-susceptibility studies were not done regularly and are therefore not included in this report.

The 56 patients selected were assigned to two chest clinics[7] and were started in May, 1957, on 500 mg. of cycloserine daily in combination with 300 mg. of isoniazid daily, the latter being continued from the previous treatment regimen. Cycloserine was given in two divided doses with the recommendation to space the capsules twelve hours apart. The products used were either combined capsules, containing 250 mg. of cycloserine and 150 mg. of isoniazid[8]; or capsules of 250 mg. of cycloserine,[9] and isoniazid tablets taken separately.

This form of medication was very well accepted by the patients, most of whom were very relieved

[1] AMERICAN TRUDEAU SOCIETY: Am. Rev. Tubere., 1957, *75*, 1016.

[2] MUON, N. D., *et al.*: Rev. tubere., Paris, 1957, *21*, 1254.

[3] BERNOU, A., *et al.*: Rev. tubere., Paris, 1957, *21*, 1173.

[4] EPSTEIN, I. G., *et al.*: Dis. Chest, 1956, *29*, 241.

[5] STOREY, P. B., AND MCLEAN, R. L.: Am. Rev. Tubere., 1957, *75*, 514.

[6] On the Pulmonary Service of Metropolitan Hospital, New York City.

[7] Manhattanville Chest Clinic and East Harlem Chest Clinic, New York City.

[8] The capsules (Seromycin®-isoniazid) were supplied by Eli Lilly & Co.

[9] Seromycin.

at not having to take large numbers of PAS tablets or to submit to twice-weekly streptomycin injections for long periods of time. In the early part of 1958, the dosage of cycloserine was increased to 750 mg. in three divided doses at six- to seven-hour intervals, in combination with 300 to 450 mg. of isoniazid daily. No other drugs, such as pyridoxine, were added at any time.

The situation in the two chest clinics already carrying a full case load did not permit the same thoroughness and precision of observation desirable in the use of a newer drug which is possible in a hospital environment with a full-time staff. Instead of objective clinical evaluation, one often had to rely on the patients' statements regarding their symptoms. Since patients were referred from many district clinics to the two centers administering cycloserine, a considerable proportion of them were not known to the medical and nursing staffs in these centers before cycloserine was started. This made evaluation of certain clinical, and especially psychologic, manifestations difficult at times.

* * *

Forty-one of the 56 patients completed one year of cycloserine therapy. Four were treated for ten months; and 7, for six to nine months. Four patients treated for less than six months were excluded from the evaluation of clinical, roentgenographic, and bacteriologic results.

Drug toxicity: As in most series of patients treated with cycloserine, the majority of side reactions were due to neurotoxicity.

Two patients developed seizures which, according to their description, were convulsions or athetotic or myoclonic movements of the extremities and face, apparently without loss of consciousness. The episodes were spontaneously described by both patients as resembling "St. Vitus Dance."

The first of the 2 patients developed the attack six weeks after cycloserine was started; the convulsive seizure allegedly lasted for several hours, during which time the patient continued to carry out some of his daily routine. He was not enough perturbed by the incident to report it to the clinic and resumed medication the next day, when another shorter attack occurred. When seen in the clinic one week later, neurologic examination was normal. Physical examination showed 2-plus ankle edema which allegedly had developed simultaneously with the convulsions. Cycloserine was discontinued immediately and not resumed again. The patient refused admission to a hospital or a hospital clinic for investigation of his ankle edema. He was admitted to a municipal hospital five months later in a terminal stage of tuberculosis and died there two days after admission. Neurologic examination was normal. There was still a 2-plus ankle edema, but death occurred before any investigation could be done; possible causes of the edema were considered by the hospital staff to be renal, cardiac, or due to "drug reaction."

It is possible that, if pre-treatment renal studies had been feasible in the clinic, this patient would not have been included in the study. This points up the desirability of having laboratory facilities easily available to chest clinics in the present era of ambulatory chemotherapy.

The second patient developed involuntary movements of the myoclonic or athetotic type a few days after increase of the daily dose of cycloserine from 500 mg., which he had received without ill effect for nine months, to 750 mg. The episode allegedly lasted for ten minutes. This patient also neglected to report the incident to the clinic and continued medication without recurrence of the symptom. When seen in the clinic three weeks later, neurologic examination was normal. Cycloserine dosage was reduced to 500 mg. and the patient completed one year of cycloserine medication without untoward reactions.

Even though the neurotoxic manifestations in these 2 patients had no apparent after effects or serious consequences, it is noteworthy that the patients did not report such rather unusual symptoms to the clinic in spite of repeated instructions to do so and good rapport with the clinic staff. This may have been partly due to a neglectful attitude, but possibly also to fear of being taken off the medication in which the patients placed much hope. This fact again points up the risk of using a new potentially toxic medication on an ambulatory basis, even in so-called cooperative patients.

A third patient became mentally disturbed in the eighth week of treatment. He complained of great nervousness, inability to concentrate, ringing in his ears, "moments of excessively serene feeling," "shimmering lights and distorted images with the eyes closed." Cycloserine was discontinued immediately. The initial symptoms subsided, but for the next six weeks he still felt "nervous and upset" and was treated by his local physician with tranquilizers which gave some temporary relief. However, his emotional state then became increasingly worse and he sought admission to the psychiatric division of a municipal hospital five months after cycloserine had been stopped.

The evaluation of this case is complicated by the fact that this 49-year-old man, who had had active tuberculosis for fifteen years, had lost his mother, with whom he had lived all his life, one month before cycloserine was started. On admission to the psychiatric ward he complained of obsessive guilty thoughts regarding his mother,

insomnia, and an inability to stay at home. Psychologic evaluation, including Rorschach testing, revealed "an emotionally unstable schizoid character with trends towards delusional thinking and autistic ideation." However, he was "cleared" psychiatrically and was soon transferred to the open tuberculosis ward where his mental condition improved markedly. Even though the patient himself attributed most of his symptoms to the drug, there seems little doubt that the death of his mother and the sudden and complete solitude which he had never before experienced were major causes for his emotional upheaval.

It is possible, however, that in this patient, with a pre-existing instability and schizoid nature, cycloserine accentuated the emotional disturbance in its early phase.

In a fourth patient, cycloserine treatment was stopped because of persistent hiccups and recurrent chills in the second month of treatment which were thought to be possibly caused by cycloserine. The symptoms stopped after discontinuance of the drug.

Thus, cycloserine was discontinued in 3 of 56 patients because of side effects. In one patient cycloserine was continued, in spite of a brief convulsive episode, with no further difficulty.

Eighteen patients had minor side effects which did not warrant discontinuance of the drug and seemed to cause only slight and transitory discomfort. The most common side reaction was sleepiness or drowsiness (11 patients). In all instances, this was described as slight and occurred within the first weeks—most often the first week—of treatment, after which time it subsided spontaneously. Transient dizziness was reported by 8 patients; increased nervousness ("shakiness") by 5 patients; this was rather pronounced in one patient who was known to the clinic as somewhat unstable before cycloserine treatment. Another patient, on the fifth day of cycloserine therapy, complained of headache, moments of disturbed memory, and anxiety. This state quickly subsided without after effects. Feverishness lasting for a few hours was reported by 2 patients during the first week of cycloserine treatment. The great majority of these side effects occurred during the first two to three weeks of therapy, some in the second month. Three of 42 patients claimed to have mild transient reactions (nervousness, dizziness) when the dosage was increased from 500 to 750 mg.

Thus, 26 of the 56 patients had toxic reactions of one kind or another during treatment (46 per cent); some patients had more than one type of reaction. Thirty patients had no reactions whatsoever (table 1).

TABLE 1

ANALYSIS OF TOXIC REACTIONS WHICH DEVELOPED IN 26 OF 56 PATIENTS RECEIVING ISONIAZID-CYCLOSERINE THERAPY

Type of Toxic Reaction	Number of Patients with this Type of Reaction*
Major toxic reaction:†	
Convulsive seizure	2
Mental disturbance	1
Persistent hiccup	1
Minor toxic reactions:	
Drowsiness	11
Dizziness	8
Nervousness	5
Nausea	2
Headache	2
Transitory memory disturbance	1
Transitory anxiety	1
Intolerance to alcohol	1
Sensory disturbance (numbness)	2
Brief febrile episode	2

* Some patients had more than one type of reaction.

† In 3 of these cycloserine was discontinued.

On the whole, frank and prolonged excitatory or depressant effects as described in many reports were not observed in this series; at least they were not obvious in the course of repeated clinic interviews throughout the treatment year. Of course, patients showed variation of mood, feeling, and behavior which are part of daily existence.

Bacteriologic results: All of the patients had sputum positive for tubercle bacilli for prolonged periods of time prior to the institution of cycloserine therapy.

Nine patients with consistently infectious sputum prior to the administration of cycloserine showed reversal of infectiousness during cycloserine therapy. In 2 additional patients whose sputum had been intermittently positive for tubercle bacilli for several years, tubercle bacilli could no longer be recovered, bringing the total number of patients who achieved reversal of infectiousness to 11 (21 per cent). The number of consecutive negative cultures ranged from five to nine in these cases. Reversal of infectiousness occurred between the first and sixth months of cycloserine therapy. No patients "converted" their sputum to negative after the sixth month of treatment. In one patient the sputum became noninfectious while he showed marked roentgenographic and clinical deterioration. The sputum "conversion" was considered equivocal in this

TABLE 2

OVER-ALL RESULTS OF CYCLOSERINE-ISONIAZID TREATMENT IN 56 PATIENTS WITH PULMONARY TUBERCULOSIS AT THE END OF TWELVE MONTHS

Bacteriologic Results	Number of Patients	Roentgenographic Results	Number of Patients	Treatment Discontinued	Number of Patients	Deaths	Number of Patients
Reversal of infectiousness	11 (21%)	Total improvement	12 (23%)	Admitted to hospital	5*	From tuberculosis	2
Improvement of infectiousness	8	Marked improvement	3	Toxicity	3*	From nontuberculous causes	2
No change in infectiousness (remained positive)	33 (63%)	Moderate improvement	4	Deaths from nontuberculous causes	2	Living at end of observation	52
		Slight improvement	5	Treatment uninterrupted	46		
		Deterioration	11 (21%)				
		No change	29				
Cycloserine continued for less than six months	4	Cycloserine continued for less than six months	4				
Totals	56		56		56		56

* Includes one death from tuberculosis.

patient and he was therefore not included in the group with reversal of infectiousness.

Three patients, while not achieving persistent reversal of infectiousness during treatment, showed a definite improvement in their bacteriologic chart. In these cases the sputum was consistently positive for tubercle bacilli before cycloserine, whereas the majority of the monthly cultures during the year thereafter were negative, with only one or two interspersed positive cultures.

Four other patients whose sputum had been consistently positive for tubercle bacilli before cycloserine therapy was started had sputum that was only intermittently positive during treatment. Thirty-three patients (63 per cent) showed no change in their sputum status, remaining infectious throughout cycloserine treatment.

Roentgenographic results: In view of the type of cases selected, the potential for roentgenographic change seemed, from the outset, rather limited. Of the 52 patients treated from six months to one year, 3 showed marked roentgenographic improvement: 2 of these had cavity closure, the size of the cavity before cycloserine being 3.5 cm. by 2 cm., and 2 cm. by 1.5 cm., respectively. The cavity closure was associated with reversal of infectiousness. The third patient showed marked clearing of infiltrations throughout the left upper lobe also accompanied by reversal of infectiousness. Moderate roentgenographic improvement was seen in 4 patients and slight improvement in 5 patients; in 8 of these cases, improvement consisted of variable diminution in the size of cavities which, however, remained patent. Two of these patients showed reversal of infectiousness.

Thus, a total of 12 patients had favorable roentgenographic changes during treatment (23 per cent). Eleven patients showed roentgenographic deterioration during treatment (17 per cent). Twenty-nine (56 per cent) had roentgenographic findings that remained unchanged.

Clinical results: The clinical changes were the hardest to evaluate accurately. Even though the attempt was made not to induce undue hope in regard to "cure" of tuberculosis with the new drug, it was inevitable that expectations of improvement were raised which, at least initially, influenced subjective symptoms in a favorable sense. Another factor working in the same direction was the close attention given to these patients while watching them for possible toxic reactions. This was particularly impressive for patients who for several years had been living with the awareness that methods of therapy for their disease had been exhausted. Another misleading factor may have been a more or less deliberate tendency in some patients to conceal untoward symptoms and overemphasize beneficial effects in order to be continued on the new medication. Considering all of these factors, and keeping in mind that improvements as well as side

reactions have been obtained with placebos in double-blind studies, the extent of clinical changes due solely to the pharmacologic effect of cycloserine is difficult to estimate accurately. Be that as it may, a considerable number of patients reported marked improvement in their general feeling and mood at a very early stage of treatment. Some patients stated they had "never felt better" in their lives; "tremendous appetite" was reported even by patients who failed to gain weight. Although there was unquestionable improvement of the general tonus and outlook in many patients, this was by no means as spectacular as has been noted in some series (except for very impressive weight gains in a number of patients).

Comparative data on subjective symptoms are not available for all of the patients. In the patients from whom sufficient data are recorded, they are as follows:

Improvement of general feeling and energy was reported by 25 of 41 patients.

Thirty-one of the 52 patients gained weight under therapy. This was often considerable, amounting to 12, 15, and 29 pounds in some cases. The average gain in weight during the year was 8 pounds, which is not negligible in view of the rather tight Department of Welfare budget on which most of the patients depended for support. Eleven patients lost weight under treatment.

Eleven of 31 patients for whom suitable data were available reported definite improvement in cough and expectoration; the actual figure is probably higher and close to 50 per cent. As in other series, some patients reported initially increased expectoration in the first weeks of treatment.

Over-all results (table 2): Five of 56 patients showed both bacteriologic improvement (reversal of infectiousness) and roentgenographic improvement. Cavity closure was present in 2 of these; in 2 others cavities decreased in size. Roentgenographic improvement not associated with reversal of infectiousness was noted in 7 patients. More than half of the patients showed improvement of one or more of their clinical symptoms. Eleven patients (21 per cent) showed either roentgenographic or clinical deterioration. Of these, 5 were admitted to hospitals during the observation period, 2 because of hemoptysis. The other 6 patients refused to enter a hospital. There were 2 deaths from tuberculosis among the entire group of 56 patients.

* * *

Insofar as conclusions can be drawn from a relatively small number of patients, it would seem that the reversal of infectiousness obtained with a rather low cycloserine dosage in 21 per cent of patients who had failed to respond to prolonged treatment with other drugs is proof that cycloserine can be useful in such cases. It remains to be seen whether the noninfectious status can be maintained during further continuation of cycloserine therapy. The incidence of 23 per cent of roentgenographic improvement is not negligible in patients with advanced tuberculosis of such long standing. To be sure, bacteriologic and roentgenographic improvement occurred only in about one-fifth of the patients. Nevertheless, the lack at the present time of another antituberculous agent with an equal, although limited, therapeutic potential gives cycloserine a definite place in the management of patients who have failed to respond to the other antituberculous drugs (streptomycin, isoniazid, and para-aminosalicylic acid). In patients who did not achieve reversal of infectiousness, the improvement of the clinical status with alleviation of asthenia and distressing cough, increase in weight, and elevation of mood, was a definite gain, since these patients had arrived at a dead end in their treatment course. Although combined cycloserine-isoniazid treatment was administered, the bacteriologic, roentgenographic and, in part, the clinical, improvements can be attributed to cycloserine with a reasonable amount of certainty since these patients had received prolonged isoniazid therapy up to the moment when cycloserine was started and there had been lack of improvement or deterioration.

In the present series, toxicity was fortunately rather low and of no really serious consequence. However, in view of the small number of patients, general conclusions as to the complete harmlessness of cycloserine should not be drawn, especially in view of higher toxicity in other series. Neither does the study imply a recommendation to start cycloserine on an ambulatory basis in patients who would be willing to enter a hospital or who would be eligible for other forms of chemotherapy, such as isoniazid, streptomycin, and PAS. The results do show, however, that definite benefit can be derived in some patients who are treatment failures and who absolutely refuse hospitalization. It is probable that with additional safeguards, such as stricter pretreatment screening, more complete pretreatment studies, closer observation than was possible in this series, and the concomitant use of pyridoxine,[10, 11] the

[10] EPSTEIN, I. G., *et al.*: Tr. Seventeenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration—Armed Forces, 1958, p. 34.

[11] EPSTEIN, I. G., *et al.*: Antibiotics Annual 1958–1959, Medical Encyclopedia, Inc., New York, 1959, p. 472.

toxic effects could have been kept at even a lower level, and larger and more effective dosages of cycloserine could have been employed.

In summary: Fifty-six patients selected from the chest clinics of the New York City Department of Health were started on 500 mg. of cycloserine daily in two divided doses, and 300 mg. of isoniazid daily, in May, 1957. For most patients, the dosage of cycloserine was increased to 750 mg. after six to eight months. The results were evaluated at the end of one year. Significant toxic reactions were observed in 4 patients. Eleven of 52 patients (21 per cent) who completed at least six months of cycloserine-isoniazid treatment showed reversal of infectiousness; 23 per cent showed roentgenographic improvement. Improvement of one or more clinical symptoms occurred in over one-half of the patients. Eleven patients (21 per cent) showed either roentgenographic or clinical deterioration.

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September 30, 1958

[1] The writer wishes to thank Dr. Aaron D. Chaves for his valuable suggestions; Drs. Zohora Ismail and Paul Auspitz of the East Harlem Chest Clinic, who participated in the planning and conduct of the study, for access to their case material; and Dr. Michael Mulinos, Director of Commercial Solvents Corporation, for arranging for the supply of cycloserine from Eli Lilly and Co.

THE "BLUING" PHENOMENON: A SOURCE OF CONTAMINATION IN CULTURES OF TUBERCLE BACILLI

Measures to control the excessive contamination rate of cultures of tubercle bacilli in the southwest area of the United States during the frequent seasonal dust storms have been described in previous papers.[1, 2] The contamination rate of 30 per cent during the dust storm season falls to 3 to 5 per cent during the remainder of the year. In these studies, two types of contamination were described with the 4,600 specimens evaluated: Type I, the presence of fungi, bacilli, and gram-negative rods which decomposed the medium; and Type II, the "bluing"[3] phenomenon, a drop in the pH of the medium from 6.6 to 3.8, and the subsequent growth of streptococci. This report is concerned with a study of the "bluing" phenomenon, and its possible mechanism.

The preparation of chloramphenicol–egg medium with concentrations of 50 γ per ml. (American Trudeau Society-C50), 25 γ per ml. (American Trudeau Society-C25), and 10 γ per ml. (American Trudeau Society-C10) of chloramphenicol added to American Trudeau Society medium[4] was described in a previous paper.[2] Sputum specimens and gastric lavages in at least 30-ml. quantity were treated with an equal volume of 4 per cent NaOH plus 1 per cent alum, and both were shaken and swirled simultaneously on an eccentric bottle shaker at room temperature for forty-five minutes. The resulting homogenized material was incubated at 37°C. for thirty minutes, then centrifuged, and the sediment was neutralized with 4 per cent HCl to pH 6.8 with phenol red as indicator. The neutralized sediments were inoculated first on one tube of American Trudeau Society-C medium and then on one tube of control American Trudeau Society medium, alternatively in successive weeks to lessen sampling errors and for statistical randomization. Cultures were read weekly for contamination and growth of tubercle bacilli, and were discarded after twelve weeks.

The data on the type of contamination found with the chloramphenicol-egg medium and the control American Trudeau Society medium[2] are analyzed with regard to the kind of specimen used (table 1).[5] Of the total of 2,117 specimens in the study, 849 (40 per cent) were sputum specimens, and 948 (45 per cent) were gastric aspirations. A marked difference in the type of contamination found in the cultures of sputum and in the cultures of gastric lavages was seen: 279 sputum specimens (33 per cent) showed Type I contamination, and 190 sputum specimens (22 per cent) yielded Type II "bluing"; only 95 gastric cultures (10 per cent) showed Type I contaminants, and 259 gastric cultures (27 per cent) showed "bluing."

The chloramphenicol in the medium was quite effective in significantly reducing the over-all contamination, regardless of whether the specimens were sputum specimens or gastric lavages. The reduction was more marked with the "bluing" phenomenon. However, sputum specimens or gastric lavages cultured on the C10 medium showed no reduction of "bluing." With C25 or C50 medium, the "bluing" was significantly reduced in the gastric specimens, but not to the same extent in the sputum.

Several questions were raised by these data: what is the nature of the "bluing" phenomenon and the subsequent growth of streptococci?; at what pH does *M. tuberculosis* fail to grow?; why should 75 per cent of the contamination of the gastric lavage specimens be of the "bluing" type?

Experiments were designed to answer some of these questions. Gram stains and subcultures to appropriate media were made of representative "blue" tubes, showing either pinpoint colonies or no apparent growth of any organism. Crude pH determinations by means of nitrazine paper were also done. Three organisms were found to be associated with the "bluing" phenomenon: gamma streptococci, nonhemolytic micrococci, (*Staphylococcus albus*), and alpha streptococci. These organisms are usually members of the normal flora

[1] GALE, D.: Am. J. Clin. Path., 1958, *30*, 177.

[2] GALE, D.: The control of contamination in the cultivation of tubercle bacilli: II. The addition of chloramphenicol to egg-containing media, to be published.

[3] Malachite green turns blue at pH lower than 6.0, and yellow at pH above 7.4, but remains green at pH 6.0 to 7.4.

[4] The modified American Trudeau Society medium prepared in this laboratory consisted of 10 per cent (approximately) potato infusion containing 4 per cent glycerin, to which was added an equal volume of egg yolk and 250 γ of malachite green per ml. of medium.

[5] Each tube was counted as a specimen, even though both the American Trudeau Society and the American Trudeau Society-C tubes were contaminated independently, one tube showing *Type I* contamination and the corresponding tube showing "bluing." Thus, the totals are not equivalent to evaluations in other tables or charts, since one specimen may have been counted twice. Such would happen with 172 of the 2,117 specimens which were contaminated on both media.

TABLE 1

THE TYPE OF CONTAMINATION WITH RELATION TO THE KIND OF SPECIMEN

Category	American Trudeau Society Chloramphenicol Medium							
	C50		C10		C25		Total	
	Number of Specimens	Per Cent	Number of Specimens	Per Cent	Number of Specimens	Per Cent	Number of Specimens	Per Cent
Total specimens evaluated	1,015	100.0	562	100.0	540	100.0	2,117	100.0
Sputum	394	38.8	233	41.5	222	41.1	849	40.1
Gastric	464	45.7	237	42.2	247	45.7	948	45.0
Urine and miscellaneous	157	15.5	92	16.3	71	13.2	320	14.9
Total sputum specimens evaluated	394	100.0	233	100.0	222	100.0	849	100.0
ATS*: Type I†	89	22.6	50	21.4	41	18.5	180	21.2
ATS-C: Type I	47	12.0	29	12.4	23	10.4	99	11.7
ATS: Type II‡	92	23.3	20	8.6	28	12.6	140	16.5
ATS-C: Type II	18	4.5	21	9.0	11	5.0	50	5.9
Total contaminated	246	62.4	120	51.4	103	46.5	469	55.3
Total gastric specimens evaluated	464	100.0	237	100.0	247	100.0	948	100.0
ATS: Type I	37	8.0	22	9.3	23	9.3	82	8.6
ATS-C: Type I	8	1.7	3	1.2	2	0.8	13	1.4
ATS: Type II	142	30.6	32	13.1	40	16.2	214	22.6
ATS-C: Type II	6	1.3	30	12.2	9	3.6	45	4.7
Total contaminated	193	41.6	87	35.8	74	29.9	354	37.3

* American Trudeau Society
† Type I: Fungi, bacilli, and gram-negative rods
‡ Type II: "bluing" phenomenon and streptococci

of the oropharynx and the nasopharynx. Blue tubes showing no organisms, at pH below 4.5, yielded streptococci on Gram stain, but no growth on blood agar plates. Blue tubes showing pinpoint colonies at pH below 4.5 yielded streptococci by Gram stain and gamma streptococci on blood agar plates. Blue tubes showing pinpoint colonies at pH 5.0 to 5.5 yielded gram-positive cocci on Gram stain and either nonhemolytic micrococci or alpha streptococci on blood agar plates. Blue tubes with nonhemolytic micrococci at pH 5.0, if incubated at 37°C. for another three to four weeks, turned yellow in patches, with pH greater than 7.5 in such patches.

The "bluing" seemed to be associated with acid production by the three organisms which were able to survive the drastic 4 per cent NaOH decontamination procedure. The following experiment was done to ascertain: (*1*) whether a nonhemolytic Micrococcus, a gamma Streptococcus, and an alpha Streptococcus could withstand the NaOH treatment and remain viable; (*2*) whether the three organisms could produce the "bluing" phenomenon on American Trudeau Society medium after NaOH treatment of the organisms.

A nonhemolytic Micrococcus, a gamma Streptococcus, and an alpha Streptococcus were isolated from representative "blue" tubes in thioglycollate medium. The growth of twenty-four-hour subcultures in the same medium were used as follows:

To three 1.0-ml. aliquots of each organism, 0.5 ml. of 4 per cent NaOH plus 1 per cent alum were added. One aliquot of each organism was incubated at 37°C. for one hour (A), two hours (B), and three hours (C), respectively.

To three 1.0-ml. aliquots of each organism, 0.5 ml. of 4 per cent NaOH plus 1 per cent alum were added. One aliquot of each organism was incubated at 37°C. as above: one hour (I), two hours (II), and three hours (III). At the end of each time interval, each aliquot was neutralized to pH 6.8 with 4 per cent HCl using phenol red as indicator (identical to the neutralization in the concentration technique for tubercle bacilli used in this laboratory).

The aliquots, A, B, C, I, II, III, and the original cultures were immediately inoculated on two tubes of American Trudeau Society medium. Similarly all aliquots were subcultured on blood agar plates and in thioglycollate broth immediately, one, two, three, four, seven, and fourteen days later. All media were incubated at 37°C.

The American Trudeau Society tubes were examined daily for fourteen days for color change

and growth of pinpoint colonies. The blood plates and thioglycollate tubes were examined one, two, three, and seven days after inoculation, for growth. pH determinations by means of nitrazine paper were done on the American Trudeau Society tubes and the thioglycollate tubes for each category. The following controls on American Trudeau Society media were also used: 4 per cent NaOH plus 1 per cent alum; 4 per cent HCl; and a noninoculated tube.

The results are summarized as follows:

A. *Subculture to blood plate and thioglycollate:*

(*1*) *Untreated organisms in thioglycollate:*

Gamma Streptococcus: grew luxuriantly in both media for seven days, despite drop in pH in the thioglycollate tubes to less than 4.5 by the second day.

Micrococcus: same results as with the gamma Streptococcus.

Alpha Streptococcus: less growth by the third day, and no growth by the fourth and seventh days. The pH of the thioglycollate subculture by the second day was less than 4.5.

It is apparent that the large amount of acid produced by metabolism and growth of the organisms by the second day was able to inhibit the growth of the alpha Streptococcus, but had no effect on the growth of the gamma Streptococcus, or the Micrococcus.

(*2*) *Treated organisms with NaOH, but not neutralized:*

No growth was obtained with any of the organisms incubated for any time interval on either blood plate or in thioglycollate. The pH of the thioglycollate tubes were all greater than 8.5, and probably 12 to 13.

(*3*) *Treated organisms with NaOH, and neutralized:*

Gamma Streptococcus: I, II, III, grew luxuriantly for the seven days, with pH drop to less than 4.5 by the second day.

Micrococcus: I, II, III, same results as with gamma Streptococcus.

Alpha Streptococcus: no growth whatsoever, pH 7.0 (same as uninoculated thioglycollate medium), for I, II, III.

These findings would imply that the alpha Streptococcus was completely inhibited by the NaOH treatment despite the subsequent neutralization. Both the gamma Streptococcus and the nonhemolytic Micrococcus could survive the NaOH treatment for three hours and, after neutralization, would grow in appropriate media despite the production of much acid.

B. *American Trudeau Society medium:*

(*1*) *Untreated organisms:* the American Trudeau Society tubes differed in their color (acid production and pH) and the growth of the three organisms.

Gamma Streptococcus: dark blue tubes (pH of 3.5) starting with the first day through the fourteenth day, with confluent pinpoint colonies from the first day.

Micrococcus: no color change and no growth on the first and second days; blue patches (pH 5.0) with increasing pinpoint growth from the third to eighth days; coalescing into dark blue tubes (pH 4.5) on the ninth to fourteenth days, with confluent pinpoint colonies by the fourteenth day.

Alpha Streptococcus: light blue tubes (pH 5.5) on the first through fourteenth days, with no growth of organisms.

(*2*) *Organisms treated with NaOH, but not neutralized:*

Gamma Streptococcus: blue patches and no growth on the first day; coalesced patches into dark blue tubes (pH 3.5) by the second day, with confluent pinpoint growth to the fourteenth day, for A, B, C.

Micrococcus: for A: yellow tubes (pH greater than 8.5) and no growth on the first day; green tubes (pH 6.5) from the second to fourth days, with no growth; blue patches (pH 5.0) with no growth from the fourth to eighth days; blue patches with 5 colonies on the ninth day, increasing to 50 colonies by the fourteenth day. For B and C aliquots, yellow tubes on the first day, and green tubes from the second to fourteenth days, with no growth.

Alpha Streptococcus: no color change and no growth for the fourteen days, for A, B, and C, (pH greater than 7.5).

(*3*) *Organisms treated with NaOH, and neutralized:*

Gamma Streptococcus: blue patches (pH 5.0) and no growth on the first day; blue patches with almost confluent pinpoint growth by the second day; dark blue tubes (pH 3.5) and confluent pinpoint growth from the third through fourteenth days, for I, II, III.

Micrococcus: no color change or growth for I, II, III.

Alpha Streptococcus: for I, no color change or growth on the first day; light blue patches (pH 5.5) and no growth on the second day; light blue tubes (pH 5.5) and no growth on the third day; light blue tubes and confluent pinpoint growth on the fourth to fourteenth days. No color change or growth for II and III.

(*4*) *Control tubes:*

4 per cent NaOH plus 1 per cent alum: yellow tubes (pH greater than 8.5)

4 per cent HCl: dark blue tubes (pH 3.5)

Noninoculated tube: green tubes (pH 6.5).

The "bluing" phenomenon thus seems to be reproducible by gamma streptococci, which could survive the strong alkali treatment for three hours, although its subsequent growth was inhibited for several days. As soon as acid was produced in its metabolism (as shown by blue patches

on the medium), growth of the organism occurred. When sufficient acid had been produced (dark blue tubes), confluent growth of the gamma Streptococcus could be seen. To a lesser extent, a nonhemolytic Micrococcus could also yield the "bluing" phenomenon, although its aciduric properties were not as marked as those of the gamma Streptococcus. It has long been accepted that enterococci (gamma streptococci) can grow in broth at pH 9.6, and can produce a final pH in 1 per cent glucose broth of 4.0 to 4.5.[6] It is, therefore, not surprising to find that the gamma streptococci can withstand the alkali treatment used in the concentration procedure for tubercle bacilli, remain viable, and be able to produce the large amount of acid demonstrable in the "blue" tubes.

The question as to what pH would inhibit the growth of tubercle bacilli on American Trudeau Society medium was explored in the following brief experiment.

Tubes of American Trudeau Society medium of different pH were prepared by adding 4 per cent HCl to aliquots of liquid American Trudeau Society medium, prior to inspissation, until the appropriate pH was reached, as checked on a pH meter. The tubes were poured and inspissated, and the pH was rechecked. No change in pH was found after inspissation. Tubes of American Trudeau Society medium at the following pH were thus available: 6.3 (control medium), 5.5, 5.0, 4.5, 4.0, 3.5, 3.0, and 2.5. The American Trudeau Society tubes at pH 3.0 and 2.5 inspissated as well as the tubes of higher pH, but were much softer. The color of the tubes ranged from green (pH 6.3), light blue (pH 5.5), blue (pH 5.0–4.0), dark blue (pH 3.5), and very dark blue (pH 3.0 and 2.5).

Three strains of tubercle bacilli, two derived from patients' specimens and BCG (Phipps), were inoculated on two tubes of American Trudeau Society medium of the various pH, as well as control medium, in 10^0 and 10^{-3} dilution. Homogeneous emulsions of the three strains were prepared in flasks with steel balls, and serial dilutions made in nutrient broth. The American Trudeau Society tubes were inoculated by pipette with 0.1 ml. of the two dilutions. All tubes were read for growth at approximately six- to seven-day intervals for nine weeks. The growth was recorded as described in the previous papers.[1, 2]

All three strains of tubercle bacilli behaved similarly, with slight variations. The inhibitory pH, beyond which no growth was observed for any strain in either concentration, was 4.5.

At eight days, patient strain A and BCG, at 10^0 concentration, showed 2-plus growth at pH 6.3 and 5.5 only, and patient strain B showed 1-plus growth only at pH 6.3. With 10^{-3} dilution, no growth was seen at any pH.

At twenty-one days, the three strains in 10^0 concentration showed confluent growth at pH 6.3, 5.5, and 5.0; 3-plus growth at pH 4.5; and no growth at pH below 4.5. With 10^{-3} concentration, patient strain A grew only at pH 6.3 and 5.5; patient strain B at pH 6.3, 5.5, and 5.0; and BCG at pH 6.3, 5.5, 5.0, and 4.5.

The pattern did not change with the three strains at 10^0 concentration for the sixty-four days observed. The growth at pH 4.5 was always less than the confluent growth in the medium at higher pH. With the 10^{-3} dilution, strain A grew only at pH 6.3, 5.5, and 5.0. Strain B grew well at pH 6.3, 5.5, and 5.0, but only after thirty-five days at 4.5. BCG in 10^{-3} dilution showed the same pattern in its growth as the 10^0 concentration.

Thus, tubercle bacilli would not grow at a pH below 4.5, and were inhibited at this pH for the three strains tested.

* * *

The question as to why 75 per cent of the contamination of gastric aspirates were of the "bluing" type does not lend itself easily to experimentation. A possible explanation, however, may be the fact that approximately 80 per cent of the gastric lavages obtained at this hospital are done through the nasopharynx, rather than through the oropharynx. The normal flora of the nasopharynx, although almost identical to that of the oropharynx, contains relatively more gamma streptococci than are found in the oropharynx. The dust storms so prevalent in this area of the United States irritate the nasal mucosa, producing a type of allergic rhinitis and a constant postnasal drip. More organisms may thus be expressed to the mucosal surface, to be carried on the surface of the Levine tube into the gastric lavage, or may be swallowed and be in the gastric contents. Since gamma streptococci can tolerate acid so well, the acidity of the stomach does not kill off these organisms. They may be aspirated by the Levine tube into the gastric lavage specimen. Another possible source of the gamma streptococci may be the backflow of the enterococci from their natural habitat, the upper part of the small intestine, into the stomach. Such enterococci may be aspirated through the Levine tube.

Some data in support of this speculation are available from other work in progress in this laboratory. The concentrated sediments from 990 specimens of sputum and gastric lavages collected from January 13, 1958 to July 11, 1958, were cultured in thioglycollate medium for forty-eight hours. When growth occurred, the constituent

[6] SWIFT, H. F.: The streptococci, in *Bacterial and Mycotic Infections of Man*, ed. 2, R. J. Dubos, ed., J. P. Lippincott Co., Philadelphia, 1952, p. 265.

organisms were subcultured and identified. Of the 158 contaminated specimens obtained, 77 (approximately 50 per cent) were gamma streptococci, 10 were lactobacilli, 24 were micrococci, 5 were alpha streptococci, and the remainder were *Bacillaceae*, *Pseudomonas*, *Escherichia coli*, *Aerobacter aerogenes*, *Penicillium*, and *Aspergillus*. The 77 specimens yielding gamma streptococci consisted of *51 gastric aspirates*, and 26 sputum specimens. The 116 specimens showing gamma streptococci, lactobacilli, micrococci, and alpha streptococci (all capable of the "bluing" phenomenon) consisted of *69 gastric lavages* (*60 per cent*) and 47 sputum specimens.

It is not surprising to find an absence of growth of tubercle bacilli on "blue" tubes, despite the presence of acid-fast organisms in smear of the concentrate, since the "blue" tubes are at pH 3.5, and tubercle bacilli do not seem to grow at pH below 4.5. If the "bluing" occurs within seven to ten days after inoculation, colonies of tubercle bacilli are very rarely seen. However, "bluing" does occur after colonies of tubercle bacilli are already present. Subcultures from such "blue" positive cultures to control American Trudeau Society medium never yield tubercle bacilli, although gamma streptococci can usually be isolated.

In summary: The "bluing" phenomenon, a drop in pH to 3.5 of egg medium, which was associated with the growth of gram-positive coccal contaminants, was found relatively more frequently in gastric lavage specimens than in sputum. It was caused by the large amount of acid formed in the metabolism of gamma streptococci and micrococci, normal inhabitants of the nasopharynx, and the oropharynx. Such organisms could withstand the alkali treatment usually used for the treatment of specimens in the isolation of tubercle bacilli, could remain viable, could produce the acid metabolically, and subsequently grow. Tubercle bacilli would not grow at pH less than 4.5 for the three strains tested. Thus, tubercle bacilli would not grow on such "blue" tubes.

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January 9, 1959

Editorial

The Natural History of Tuberculosis in the Human Body
II. Longitudinal Observations Imperative[1]

When one is concerned with the evolution in the human body of a disease which acts swiftly and with finality, such as most forms of cancer, only a few years of observation are necessary of a given group of patients to determine at least the later part of its natural history. On the other hand, a disease that may be lifelong among people, causing no significant illness in many, maiming others, and killing some, can have its entire natural history determined only by keeping under observation a group of people who acquire the disease at various ages as long as they live.

Reference has often been made to the importance of long-term follow-up observation of patients. W. C. Alvarez[2] says, "The only trouble is that a busy practitioner is usually so overwhelmed that he cannot stop to write the wonderful book of prognosis for which he has so much excellent material. Commonly, also, he does not realize what an opportunity he has to make a real contribution to medicine."

Paul D. White[3] said:

> It was through the correction of errors in the course of follow-up studies that my associates and I were able to contribute new data concerning pulmonary embolism and the acute cor pulmonale, chronic constrictive pericarditis, the WPW electrocardiographic syndrome, coronary heart disease, and the range of the normal heart.
>
> One of the weakest aspects of our medical practice today is that of prognosis, in large part because of its difficulties and of our failure to spend enough time on its background. . . . I want to emphasize the tremendous importance of *the long follow-up*, an opportunity and an obligation of the practicing physician. One of the defects of medical teaching in recent decades has been the lack of adequate follow-up by some of the heads of medicine themselves, many of whom, of course, have lived a somewhat peripatetic life or have not had time to devote to this particular aspect of medicine. Part-time teachers of medicine who are in active practice and general practitioners, therefore, have an important obligation which they have not adequately utilized. Most of the clinical research data that have been recorded are concerned with short-time studies.

Dr. White pointed out that a follow-up period of twenty-five years or more of 200 cases of severe coronary thrombosis seen in the decade from 1921 to 1930, inclusive, has been of great value in prognosis.

Kinsella[4] called attention to plombage and extrapleural operations, which were so popular approximately two decades ago. He did sixty-five such procedures between 1934 and 1939. For five years the patients appeared well and were active, but fifteen to twenty-two years later not more than a half dozen had avoided serious complications which required treatment. It was only this longer period of observation that afforded the facts and gave him the courage to reverse his stand and publish later results that should be helpful to other professional workers. This study will not be complete until the remaining half dozen have been followed through life.

Apparently the most extensive and thorough long-term follow-up medical study was done by Gjestland[5] on the Boeck-Bruusgaard[6] material on untreated syphilis.

> Between 1891 and 1910 Boeck hospitalized approximately 2,000 persons with primary and secondary syphilis until lesions healed without treatment, believing that the patient's own defense mechanism alone could better combat the disease than the antisyphilitic treatment of the day.

Gjestland[5] seized the opportunity of determining prognosis in these untreated cases in 1953–1954 because:

> . . . the prognosis of untreated syphilis is not accurately known. Long-term studies of patients treated in all stages of syphilis attest to the beneficial effects of treatment in modifying the biologic course of syphilis infection, but what happens to those who go untreated is unknown from a quantitative and comparative standpoint. Kampmeier expressed his opinion on this point as follows: 'It would be of great value if the prognosis in untreated syphilis were accurately known. As was indicated . . . , this is not known and probably never will be known in these days of more or less universal treatment of the disease.'

[1] Prepared with the aid of the H. Longstreet Taylor Research Fund provided by the Minnesota Tuberculosis and Health Association.

[2] ALVAREZ, W. C.: Geriatrics, 1956, *11*, 470.

[3] WHITE, P. D.: Dis. Chest, 1957, *32*, 368.

[4] KINSELLA, T. J.: Dis. Chest, 1957, *31*, 525.

[5] GJESTLAND, T.: Acta dermat.-venereol., 1955, *35* (Supplement 34, p. 1).

[6] BRUUSGAARD, E.: Arch. f. Dermat. u. Syph., 1929, *157*, 309.

Gjestland's study was a tremendous undertaking and is an excellent example of international cooperation not only of individual workers and universities, but also of nations, since the Norwegian Public Health Service and the United States Public Health Service participated.

This follow-up study of persons for as long as sixty years resulted in the first authentic account of prognosis in untreated syphilis which could have been obtained in no other way.

Clarke and Danbolt,[7] after a careful review of this work said: "Gjestland's contribution will stand as a model of carefully planned and successfully executed field research. . . ."

There is serious sparsity of long-term follow-up studies of persons with tuberculosis in various stages of its evolution in the human body. Therefore, prognosis among those who have no evidence of tuberculosis except the tuberculin reaction when first seen, those not ill at the time progressive disease is discovered, those who have survived an attack of clinical disease, et cetera, has not been well established.

Sir Robert Philip,[8] who started the first dispensary for consumptives in the world at Edinburgh in 1887, had a thorough appreciation of the value of follow-up work among tuberculous patients and accumulated extremely valuable information which he could not have obtained in any other way. In 1921, writing about advances in the knowledge of tuberculosis, under the heading "Continuing Observations," he said:

> This advance has been largely contributed to by prolonged, continuous observation of individual cases—not for weeks or months only, but through many years—from the earliest manifestation, for example, in an isolated gland, to successive further developments throughout the subject's life. I have in this manner followed numerous patients from early childhood to manhood or from early adolescence throughout adult life. The line of study is tedious and difficult, but throws a flood of light—not otherwise available—on the natural history of tuberculosis as a whole and particularly on that aspect of the subject which we are now considering. I commend the method of observation to younger workers, who presumably have the potential of carrying it out for sufficient periods.

Knowing about Sir Robert Philip's longitudinal studies and the large volume of information he had gained from them prior to 1920, the idea was conceived of trying to make similar observations in this country. The opportunity to institute such a project came in 1920 when observations began on tuberculosis among university students, who by 1956 totaled more than 160,000. About the same time, studies were started on private patients with a subsequent accruement of more than 6,000. Also in 1920, work began on persons of various ages admitted to tuberculosis and chest clinics and who over the years numbered several thousand. In 1921, observations were instituted on tuberculosis among children, whose number finally totaled more than 19,000. As the years passed, several hundred persons were examined for silicosis.

About a decade ago it was thought that, with such numbers of persons in all ages of life and engaged in so many activities, efforts to trace and obtain health data from them would yield important information concerning tuberculosis and closely allied conditions. Moreover, it had become obvious that a study of the natural history of tuberculosis would be possible only on persons whose observations began before the antituberculous drug era was well established.

* * *

On request in 1950, Dean H. S. Diehl and chiefs of the various departments of the School of Medicine at the University of Minnesota attended a meeting to discuss a follow-up project in tuberculosis and decide whether it was the type of research they could approve and wholeheartedly recommend. There was unanimous agreement in its favor.

It was obvious that such an undertaking would require sizable funds and a large amount of time. Dr. W. F. Braasch of the Mayo Foundation, Dr. Irving McQuarrie of the School of Medicine, University of Minnesota, and other members of the Executive Committee of the Minnesota Tuberculosis and Health Association presented a motion to the effect that the organization make an annual grant for research at the University of Minnesota. This motion received a unanimous vote which also met with the approval of Dr. E. A. Meyerding, Executive Secretary of the Minnesota Tuberculosis and Health Association. The research fund was established in memory of Dr. H. Longstreet Taylor who pioneered the early campaign against tuberculosis in Minnesota, beginning in 1893. It was designated the "H. Longstreet Taylor Tuberculosis Research Fund" and it was specified that the fund be presented to the University annually for follow-up studies in tuberculosis.

Follow-up work was not a new experience as we had traced several groups of former examinees and patients in earlier years. Our first such study

[7] CLARK, E. G., AND DANBOLT, N.: J. Chronic Dis., 1955, *2*, 311.

[8] PHILIP, R.: Tr. Tuberculosis Society of Scotland, 1921–1922, p. 4.

was on children, the first of whom were examined in 1921. The result was published in 1935.[9] A second survey of the children in this study group was published in 1944.[10]

The first follow-up study on students of nursing included those whom we had examined in several schools beginning about 1927, and the results were published in 1940.[11] In one school of nursing, another follow-up study was done in 1946 and was published in 1947.[12]

Among former students of medicine, the first follow-up study included the classes of 1919 to 1936. The results were published in 1941.[13] Former Medical School students were again traced, beginning with the class of 1933 and ending with the class of 1947, and the results were published in 1948.[14]

In 1943 a report was issued on a follow-up study on tuberculosis among former students of our School of Law.[15] This was done because considerable controversy had arisen concerning the prevalence of tuberculosis among students and graduates in medicine. It had been contended that students and graduates in professions other than those engaged in health work develop tuberculosis as frequently as do those of the medical profession.

Since the H. Longstreet Taylor Research Fund became available, more extensive follow-up studies have been done. Efforts to trace 300 persons (among more than 19,000) who as children had demonstrable primary tuberculous pulmonary infiltrates resulted in finding 267.[16] The average age of these children when first examined was six years for girls and five for boys. Of the 241 traced and living in 1958, the women were 32.3 years and the men 30.9 years old. The 231 survivors who had been recently examined had 6,009 person-years of follow-up observations. Various groups of the remainder of the 19,000 former children are now being traced.

A follow-up study of former students of nursing in several hospitals, beginning with the classes of 1930 and ending in 1951, included 4,633 graduate nurses, 4,030 (87 per cent) of whom were traced. The findings in this study were published in 1955.[17]

The results of the last follow-up study on former students of medicine were published in 1955 and *included* the classes graduating from 1930 to 1951, *including* 2,683 physicians, 99.3 per cent of whom were located.[18]

In 1954, a report was issued on a follow-up study of 115 cases of simple spontaneous pneumothorax. Those traced had been observed from one to twenty-nine years. Of these, 104 were located.[19]

In 1955, a report was published on the follow-up observation of 260 cases of tuberculous pleurisy with effusion in the absence of demonstrable clinical pulmonary disease.[20]

When our observations on tuberculosis were begun in the early 1920's, the diagnostic armamentarium included conventional history-taking and physical examination. The tuberculin test and the roentgenogram were in use. Bacteriologic studies consisted of stained smears of sputum, gastric washings, urine sediment, pus from discharging cutaneous sinuses, et cetera. Specimens for pathologic studies were obtained post mortem, and biopsy of suspected available tissues, such as peripheral lymph nodes, was often employed. Animal inoculations were often the final step in diagnosis.

Later in the course of our studies, biopsy material, consisting of tissues and aspirated tracheal and bronchial contents, was obtained by bronchoscopists; finally, biopsy tissue specimens of pleura, lungs, et cetera, were obtained as indicated by the surgeons.

In the early 1920's, treatment consisted mainly of bed rest, preferably in sanatoriums. In the area where most of our work has been done, pulmonary collapse was beginning to get under way. In fact, in institutions it was not unusual for 50 to 65 per cent of the patients to be on pulmonary collapse treatment. Beginning about 1945, a few patients were treated with streptomycin. As this became more available and other antituberculous drugs were introduced, they were employed in nearly all cases of both pulmonary and extrathoracic tuberculosis. Soon after the suppressive effect of drugs was assured, resectional surgery began to play a considerable role in treatment.

In our follow-up work, therefore, it has been important to give careful consideration to situa-

[9] Myers, J. A., *et al.*: Am Rev. Tuberc., 1935, *32*, 631.

[10] Harrington, F. E.: The Evolution of Tuberculosis as Observed for Twenty Years at Lymanhurst, Minneapolis Board of Public Welfare, Special Publication, 1944.

[11] Myers, J. A., *et al.*: Ann. Int. Med., 1940, *14*, 873.

[12] Myers, J. A., *et al.*: Am. J. Nursing, 1947, *47*, 661.

[13] Myers, J. A., *et al.*: Ann. Int. Med., 1941, *14*, 1575.

[14] Diehl, H. S., *et al.*: J.A.M.A., 1948, *134*, 8.

[15] Myers, J. A., *et al.*: Yale J. Biol. & Med., 1943, *15*, 439.

[16] Myers, J. A.: Am. Rev. Tuberc., 1959, *79*, 19.

[17] Myers, J. A., *et al.*: Dis. Chest, 1955, *28*, 610.

[18] Myers, J. A.: Tuberculosis in physicians, J.A.M.A., 1955, *158*, 1.

[19] Myers, J. A.: Dis. Chest, 1954, *26*, 420.

[20] Myers, J. A.: A.M.A. Arch. Int. Med., 1955, *96*, 191.

tions and procedures in vogue at various times from 1920 to the present. For example, earlier diagnosis and administration of antituberculous drugs in the past decade have resulted in marked change in at least immediate prognosis.

* * *

Inasmuch as follow-up studies on former examinees and patients have been employed for about twenty-five years and have included several thousand persons, experiences are cited relating to problems which often resulted from oversight, neglect, inadequate records, and the like, in order that such pitfalls may be avoided by others who engage in follow-up work. On the basis of these experiences, attention is called also to procedures by which others may profit.

At the time of the original examination or treatment, it is important that a careful record be made including the first and middle names of the individual along with complete names and addresses of parents, if living, and especially of brothers. The individual's religion and name of the church of membership should be recorded. Maiden names and places of birth of married women should be entered on the chart. When there is no close relative, names and addresses of at least two personal friends should be obtained. Names and addresses of all former physicians should be included.

When we began to trace persons who had not been seen for many years, letters were sometimes found in charts containing no dates except perhaps "Monday night" or "Tuesday morning." When such letters are received, the physician or his secretary should add at least the month and year. Another unfortunate practice is filing newspaper clippings concerning patients without date or place of publication.

Chest roentgenograms are an important part of follow-up studies. The date of exposure and the individual's name should be imprinted in large letters and figures on the film. Fine type or script consume a great deal of time of the person who is making comparisons of numerous films exposed at different times over a period of many years. In some places, the date of exposure and the patient's name are written on a paper label which is pasted on the film. These often become loosened and torn or completely lost, leaving no film identification.

Again, names and dates are written on roentgenograms with ink or colored crayon so illegibly—with black ink for example over the dark part of the film—that they are exceedingly difficult to find and read. Again such identifications become rubbed off. On occasion we receive films with no name or date. There may be a number on the jacket which means nothing to anyone except those working in the office of the physician who exposed the film, where a list of the patients' names and the numbers of their films are kept separately. Such lists are sometimes lost, the physician has died or moved away, and all records have been destroyed.

Good technique is of utmost importance in roentgenography. In follow-up studies, all too often when films of the same person's chest are assembled from various places over a period of years, too little or too much exposure and other factors make them technically so different that they are of little value in following the evolution of the disease.

After films are developed, the washing process must be thoroughly done to make certain that all developer has been removed. If not, celluloid films deteriorate over the years to such a degree that they are worthless. We have lost some of our most precious records by failure on the part of X-ray technicians to adequately wash films.

Preservation of films is exceedingly important in all follow-up work. In the past, many departments of radiology in hospitals and clinics, and even radiologists and other physicians in private practice, preserved films for only a brief time (usually not exceeding five to seven years), after which they were sold for junk or destroyed. This was an unfortunate practice because, at any time during the patient's life, films previously made may become of great value to the clinician and the investigator. It was argued that reports of readings of roentgenograms were sufficient, but for follow-up work this is not true. In viewing a roentgenogram, it is impossible to describe all that one sees so that another person or even the same person can later visualize it. Everyone who reads films occasionally overlooks small shadows or may not consider them significant and, therefore, makes no mention of them in the report. When such shadows are later proved to represent significant lesions, it is important that the previous films be available to aid in the study of the evolution of the disease. Many persons with clear films on an initial examination may subsequently develop significant shadow-casting lesions, and the physician desires to inspect the previous films since they give him some idea of the duration of the disease process. In our follow-up work, we have been fortunate that a few hospitals, sanatoriums, and private physicians have preserved films indefinitely. Every place where roentgenograms are exposed and developed should be encouraged to preserve them as long as there may be need for them in follow-up work.

It is also important that hospitals, clinics, and private physicians preserve patients' charts. The hospital chart may be the only record the phy-

sician has. For example, in a private hospital where a sizable number of our patients were institutionalized and treated during the 1920's and early 1930's, we had no reason to suspect that charts would not always be available, at least for the lifetime of the physician who referred the patients. However, years later when these charts were requested as a part of one of our follow-up studies, we were informed that they had been destroyed. Thus, not only a large volume of work, but valuable information and a considerable segment of our follow-up study were lost. When these charts were scheduled for destruction, the physicians who referred the patients should have been notified.

In many hospitals, preservation of charts poses a problem because of space requirements. A few years ago, a wave of enthusiasm for microfilming of charts made follow-up work with patients' records exceedingly difficult and in some instances impossible. To microfilm a patient's chart is only a little better than to destroy it. Once its pages are microfilmed, they must be viewed with special magnifying equipment which means they cannot be taken to the physician's home or office for study at especially convenient times. Often handwriting is not legible when microfilmed. The time consumed in going over a microfilmed chart is so great as compared with reading the original as to discourage such effort. Moreover, reading microfilmed charts is especially tiresome, particularly for the eyes.

* * *

The physician who is starting a professional career will do well to contact his examinees and patients annually. If sufficient effort is made, a high percentage of persons will respond to inquiry each year and those who do not by reason of having moved, et cetera, are usually easily re-contacted. A good method is to send a personal birthday letter or a holiday greeting. The individual is impressed with the physician's continued interest and is likely to respond to annual requests concerning health.

The follow-up procedure we have used most consists of mailing to each person included in a study group a questionnaire introduced by a statement concerning its purpose. These have usually brought about a 40 per cent response. For the remaining 60 per cent, in due time a personal letter is mailed which usually nets a good yield, although several letters may be necessary. In some cases, we have mailed registered letters with request for return receipt.

When letters are returned from former children's home addresses, it has often been found that the parents have died or moved. In such cases it has been reasonably fruitful to contact *neighbors* by calling the house numbers adjacent to and across the street from the old home. Many persons called in this way have been helpful.

When unable to locate the family or a former neighbor, we have sometimes contacted *clergymen*. Those who have served the same churches for a long time have in some instances kept in touch with the families or have provided addresses of those who knew them. Occasionally, clergymen have gone through old records and procured valuable information. On a number of occasions, letters or telephone calls to *village* or *city clerks* have brought addresses, married names, et cetera, of persons born there or who may even have resided there for only a short time. In contacting neighbors, clergymen, city clerks, and others, we always try to make it clear to them, as well as to the former patients themselves, that all information will be held in strict confidence and that no name or identifying statement will appear in any report. Some *business concerns* keep records of former employees only five years after they leave.

A good deal of difficulty was encountered with women *changing names* through marriage. For example, those who were only school girls when we last saw them were difficult to locate, particularly if their parents had died or moved away. Occasionally, even men had changed their names.

Departments of vital statistics in municipalities and state boards of health are often excellent sources for determining whether certain persons who are being traced have died and, if so, the date and cause of death are made available.

Telephone and city directories have been found of great help for those whose names have not been changed and who are residing in the same city where the initial examination was made. When the first names and initials are available for the individual who has been lost through death or moving, it has been helpful to call all persons of the same last name listed in the directory. There are usually not more than twenty to thirty of the same name in a directory. However, in the area where we have worked, there are so many Scandinavian names, such as Anderson, Olson, and Peterson, that it is almost an impossibility to call all with the hope of finding relatives. For example, in the Minneapolis telephone directory issued January, 1958, there are 4,242 listings under the name of Anderson, 2,354 under the name of Olson, and 2,266 under the name of Peterson.

The Business Library of the city of Minneapolis carries a large number of directories of other cities. The employees of this library have been very helpful in providing us with street addresses and telephone numbers of "lost persons" residing in other cities. If letters are not claimed or are un-

answered, we have communicated with a physician friend residing in that city. For example, a letter to Dr. J. E. J. Harris, Albuquerque, New Mexico, resulted in his locating four persons of one family at four different addresses (not all in Albuquerque).

In tracing former students of medicine, the *Directory of the American Medical Association* was exceedingly helpful. The American Medical Association has been a dependable source for date and cause of death, as well as residence at the time, of a considerable number of physicians.

In tracing former students of nursing more difficulty is encountered since there is no nationwide directory. However, Miss Leonora J. Collatz, R.N., Executive Secretary, Minnesota State Board of Examiners of Nurses, has cheerfully provided us with a large volume of important information as to the whereabouts of former students, including those who have left this state.

When correspondence has failed, we have used long-distance telephone. A prominent physician practicing in New York City developed tuberculosis as a student in our School of Medicine about 1934. His address was readily obtained and a considerable number of letters were written. None was returned nor did he reply. When called on the telephone at his office in New York, he was most apologetic, gave the requested information, and stated that procrastination was responsible for his failure to respond.

When we were tracing former students of medicine, there were on the "no-response list" about two dozen practicing in California. While attending a medical meeting in that state, it was possible to reach each one on the telephone. In addition to periods of enjoyable reminiscence, all of the desired information was obtained.

All contacts with persons being followed or traced must be kept on a *friendly basis*. This can be done by manifesting sincere personal interest in their health, and not becoming irked or manifesting displeasure with those who have failed to respond promptly. A demonstration of impatience can close the door permanently. The occasional person has had so many unfortunate health experiences and so many requests for health information as to make more unwelcome.

Indirect misinformation can cause embarrassment. Letters had been sent to a former student nurse after she had changed positions. Finally a statement reached us from an indirect source that she had died in a southern city. A letter was sent to the official nursing organization of that city and a reply arrived with the opening sentence "I am very much alive." She then cheerfully gave us complete health information. Most persons followed expressed appreciation of the interest being manifested in them and willingly cooperated.

Careful follow-up work requires patience which makes that manifested by Job seem puny. Concerning follow-up information on cases of proved carcinoma of the lung, Rigler and associates[21] say:

> Letters were written to patients, wives, relatives, family doctors, surgeons, hospitals, and Public Health agencies begging for information. Of 142 such letters, 75 were answered, and of these, 29 produced material of use or interest. Frustrating factors were many. Vagrancy made continued observation impossible in some cases. With the death of the patient the wife usually moved, often without leaving a forwarding address, and sometimes she kept moving, living first with one of her children, then another. Some of the wives had died, while others had difficulty with senescent memories. The family doctor often had no X-ray films or had sent them to a surgeon or hospital. Surgeons and hospitals often returned their films and records to their point of origin so that some seemed forever in transit. Some large clinics destroyed their roentgenograms. Perhaps the greatest disappointment came from the Public Health and mobile chest survey organizations who sometimes destroyed but more often lost the records, apparently through administrative difficulties.

A graduate nurse received a letter of inquiry every six months for two years with no response. The fifth letter brought a splendid report and she returned four stamped envelopes we had previously sent her. In our follow-up of nurses for whom we had no recent address, inquiries were usually sent to them at the home of their parents. Some of those who were married replied, often from different places, giving us wonderful information but using their married names without mention of their maiden names or from what school they had graduated. This left us helpless until the number of untraced cases was so narrowed that we could start solving the riddles.

Another serious handicap was when local health departments decided to close cases with no further follow-up study when no reactivation or new lesions appeared within two years after treatment.

Sanatoriums have been one of our best sources of follow-up information with reference to status on admission and discharge, treatment administered, and often good subsequent data. However, that source has diminished markedly in the last few years with the closing of many sanatoriums and the loss of ready access to their records.

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[21] RIGLER, L. G.: Dis. Chest, 1953, *23*, 50.

We have had considerable trouble tracing former tuberculous patients whose names were dropped from health department follow-up lists. They have expressed great fear of being returned to the lists and, therefore, have refused to provide complete and, in some cases, all information.

For persons who have moved to distant points, their private physicians, hospitals, sanatoriums, and clinics have been most kind in administering tuberculin tests, making and forwarding new roentgenograms, and providing other information. Some physicians have taken the time to write long letters including details about the individuals' health since we last saw them.

The occasional person becomes completely lost to family and friends. For example, after numerous attempts to trace a former medical student, his classmates were contacted. One replied, "Unfortunately, he became a confirmed alcoholic, he divorced his wife, tried one job after another, and finally disappeared completely. As far as I know, nothing has been heard of him for about ten years." Another classmate wrote that he was last seen on Skid Row several years ago in Chicago.

* * *

In 1933, D. A. Stewart[22] wrote: "How long should you follow up the child who has minimal healed disease?" An experienced tuberculosis worker was asked this question. "Until he dies of old age" was the reply. This can now apply to all tuberculin reactors.

If one does follow-up work on a group of examinees or patients for brief periods, such as three to five years, all statements concerning findings should be emphatically qualified with reference to duration of the follow-up observations. Otherwise, readers may assume that they apply to the remainder of the lifetime of the persons concerned. Only continued observation over the years can provide the actual reactivation or new lesion rates. Obviously, each year of follow-up study enhances the value of a study as it brings individuals nearer the end of the span of life. Therefore, it is our intention to survey our various groups of people about every three to five years.

To date, follow-up work on children has been rewarding. Facts came to light contrary to previous generally accepted opinions.[9,10,15] For example, roentgenograms of chests of children who were nonreactors to tuberculin never revealed evidence of chronic pulmonary tuberculous lesions. Thus, such lesions were detected only in the chests of tuberculin reactors. However, even in tuberculin reactors chronic clinical lesions were so rare before the period of adolescence as to justify the recommendation that periodic roentgenograms of the chest be withheld until about the ages of twelve to fifteen years.

Primary tuberculous pulmonary infiltrates were observed to be so benign that they rarely caused significant symptoms and were not influenced by methods of treatment available at that time. Clinical tuberculosis which was then so destructive among teen-age girls and boys developed in those who were previously reactors to tuberculin. Thus, such cases were not rapidly progressive primary disease occurring in those in whom first infection was postponed to the teen-age period, as had previously been suspected. It was also observed that it is imperative to differentiate between primary tuberculous pulmonary infiltrates and pulmonary lesions of the reinfection type in order to avoid erroneous deductions concerning the value of treatment procedures and methods designed to prevent tuberculosis. The follow-up study on 19,000 former children is still in progress, but nothing has come to light to nullify the validity of the early observations.

Among student nurses, follow-up study revealed that those in whom infection is postponed until adulthood develop the benign primary type of tuberculosis which behaves in the same manner as when it first occurs in children. It was also observed during the early years of this project that the tuberculosis hazard among student nurses was much greater than that among girls of the same age period in other activities. However, methods were developed to correct or abolish dangerous situations. It also became evident that no method available, except rigid contagious-disease technique, prevented the spread of tubercle bacilli from patients to students. During this period of follow-up observation, contagious-disease technique was established, and later observation revealed that the former tuberculosis hazard among student nurses had been reduced to a trickle.

The opinion that nearly all student nurses become infected with tubercle bacilli while in school and the remainder soon after graduation was not confirmed. In fact, among 4,633 there were 2,441 who graduated uninfected, only 414 of whom became infected throughout the period of follow-up study after graduation.

Those who developed chronic clinical tuberculosis while in school or after were previous tuberculin reactors rather than nonreactors, as previously had been contended. The follow-up study revealed that approximately as many clinical lesions evolved after as during the first two-year period after infection occurred. A considerable number appeared ten or more years following first infection. These findings justify

[22] STEWART, D. A.: Canad. Pub. Health J., 1933, *24*, 269.

the recommendation that all adult tuberculin reactors be examined periodically as long as they live.[11, 13, 17]

A follow-up study of former medical students reported in 1941[13] revealed that, among 90.6 per cent traced in the graduating classes of the University of Minnesota from 1919 to 1932, demonstrable tuberculous lesions developed in 92 (7.1 per cent) while in school or after graduation, 11 of whom died from tuberculosis.

Another follow-up study reported in 1955[18] was limited to those who graduated from 1930 to 1951, inclusive. Of the 2,683 graduates in these classes, 2,665 (99.3 per cent) were located.

The last follow-up data revealed that 33 of the 1,021 who had been infected before entering medical school had developed clinical tuberculosis. Among 968 who became infected while in school, clinical disease had evolved in 25. The belief that nearly all physicians uninfected with tubercle bacilli on graduation will soon be infected and that 10 per cent or more will be ill from clinical tuberculosis within five years after leaving school was not substantiated. Among 1,218 uninfected at the time of graduation, 491 had become tuberculin reactors, and clinical lesions had appeared in only 0.66 per cent of the 1,218. Just as among nurses, follow-up study revealed that primary lesions took the same benign course as in children, and chronic clinical disease appeared among the previously known tuberculin reactors.

The follow-up study on 1,209 who had graduated from Law School from 1919 to 1936, inclusive, resulted in response from 83.64 per cent. Only 1.33 per cent of the attorneys had developed clinical tuberculosis while in school or after graduation as contrasted with 7.1 per cent among physicians.[12]

In summary: The importance of longitudinal observations on persons with various disease conditions has been emphasized by many physicians. Sir Robert Philip of Edinburgh, Scotland, was the first to report on long periods of follow-up study on persons with tuberculosis.

Knowledge of the natural history of tuberculosis in the human body is not complete because no sizable group of persons infected in childhood has been followed through the span of life.

Attention is called to longitudinal studies made of tuberculosis in persons of all ages of life beginning about 1920. It is recognized that final conclusions are not possible at the end of thirty-eight years inasmuch as the average span of life in this country is approximately seventy years.

Since many more follow-up studies on tuberculosis as well as on various other diseases should be made, attention has been called to procedures employed over this period of years which may be useful to others undertaking such projects. Some accomplishments resulting from follow-up procedures are presented.

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Correspondence

CHEMOTHERAPY TO PREVENT RELAPSE IN PATIENTS WITH NONINFECTIOUS TUBERCULOSIS

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

During the past year it has become obvious that there is a gap in the complete use of drugs for treatment of tuberculosis. Chemotherapy is used for a wide variety of indications in which bacterial specimens have been positive for tubercle bacilli. It is used for long-continued therapy; for diseases which have become "inactive"; and for the "open-negative" syndrome. It is used for new infections, whether or not the lesions can be demonstrated by X-rays ("converters" or primary disease).

The additional situation in which chemotherapy could be used with potential profit is in cases with negative bacterial findings. It would be aimed at the prevention of relapses which should be inexcusable in this era, but which are seen repeatedly in private practice, in the admissions to sanatoriums, in public health clinics, et cetera.

Six months ago it was suggested to several colleagues in the Los Angeles area that a new attitude should be taken in this situation. The idea was also presented in the past two months at the Chest Disease Symposium in Los Angeles and at the Pacific Western Tuberculosis Conference of the Veterans Administration. It was agreed that the plan was logical; that some physicians were occasionally using it; but that no one knew of an outright recommendation on the subject. It was suggested that this letter be written to describe the local attitude and, perhaps, to crystallize a latent approval into a strong stand by more physicians.

The program would involve the use of isoniazid alone, probably in doses of 100 mg. three times daily, for a period of one or more years. The indications would include the following: (*1*) All patients now being observed (sometimes over a period of years) in private practice or public health clinics who are considered to be tuberculous by reason of X-ray, skin-test findings, et cetera, but who have been bacterially "inactive" by regular or occasional tests; (*2*) all patients who once had clinical tuberculosis but who are now "inactive" and who have never had chemotherapy; (*3*) all patients who have newly discovered lesions by X-ray, presumed to be tuberculous by thorough examination, but which are bacterially negative for tuberculosis.

The successful initiation of this program would require general medical approval, since experimental proof of the efficacy would take years to obtain. Its value would have to be presumed from our present knowledge of drug effects. The patients would have to be assured that it was a preventive method; that they would have to be under occasional medical observation; that the toxicity and complications would be very rare; and that the use of the drug would be simple and inexpensive. Occupational, insurance, and other aspects of the problem would have to be convinced that the objective was prophylactic and helpful.

I am sure that, since the plan has occurred to me, many others have also considered and used it. I can only hope that the possible value of wider usage will compensate for any redundancy.

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March 17, 1959

FURTHER OBSERVATIONS ON THE CORRELATION BETWEEN ISONIAZID SERUM CONCENTRATIONS AND THERAPEUTIC RESPONSE IN HUMAN PULMONARY TUBERCULOSIS[1]

To The Editor of The American Review of Tuberculosis and Pulmonary Diseases:

At the 1959 Veterans Administration-Armed Forces Conference on the Chemotherapy of Tuberculosis in St. Louis, J. H. Peters of Cincinnati reported a new spectrofluorometric method for the measurement of free isoniazid in *plasma*. Results with this chemical method compared well with bioassays for free isoniazid on duplicate specimens. Six of nine human subjects showed only small rises (up to 0.7 γ per ml.) in their two-, four-, six-, or eight-hour plasma isoniazid concentrations following the addition of 4 gm. of sodium para-aminosalicylic acid (PAS) to the oral test dose of 4 mg. of isoniazid per kg. of body weight.

We have reviewed our experience with 317 patients tested at two and six hours following a 4-mg. of isoniazid per kg. test dose with and without 5.5 gm. of sodium PAS (table 1). It will be noted that 36 per cent showed *no* change (but could actually have had some change (table 2)) and 8 per cent showed a drop averaging 0.75 γ per

[1] Supported by a contract (#SAph 70474) with the U. S. Public Health Service; and by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

TABLE 1

INFLUENCE OF 5.5 GM. OF SODIUM PAS ON SIX-HOUR SERUM ISONIAZID BIOASSAY CONCENTRATIONS

Isoniazid (4 mg./kg.) Alone at 6 Hours in γ/ml.	Total Number of Patients	Number of Patients										
		Drop to					No Change	Rise to				
		1.6	0.8	0.4	0.2	0.2		0.2	0.4	0.8	1.6	3.2 γ/ml.
<0.2	85						30	22	26	3	4	0
0.2	55					7	11		24	10	3	0
0.4	33				3	2	6			17	4	1
0.8	89			0	0	1	37				49	2
1.6	49		6	2	0	0	28					13
3.2	6	3	0	0	0	0	3					
Totals..	317	24 8%					115 36%	178 56%				

ml. One hundred and seventy-eight or 56 per cent showed a detectable rise averaging 0.9 γ per ml. If all rises had been the lowest possible (table 2), the mean rise would have been 0.26 γ per ml. in this group. In short, the rises in *serum*-free isoniazid (using the bioassay and a slightly larger dose of sodium PAS) were of about the same order as noted chemically by Peters.

At the same meeting, W. R. Barclay, in discussing a paper on isoniazid serum concentrations and their possible relationship to bacteriologic results with isoniazid-containing regimens, reminded us of the early binding of free isoniazid by H37Rv in *in vitro* systems. He speculated, therefore, whether a one- or two-hour serum concentration might not be more apt to show correlation with therapeutic results than six-hour concentrations.

In table 3, two series of patients with advanced, cavitary, drug-susceptible, pulmonary tuberculosis are presented: 30 who received high isoniazid-PAS and 60 who received high isoniazid-PAS plus streptomycin daily for at least ninety days. "High" means at least 12 mg. of isoniazid per kg. Streptomycin dosage was adjusted by age, renal function, and/or serum concentrations. All persons received isoniazid-PAS for at least twelve months. Reversal of infectiousness seems to have occurred somewhat more in relation to six-hour than to two-hour concentrations, we believe, although the differences are not statistically significant.

In summary: On the basis of approximately 400 patients studied retrospectively, we believe that isoniazid inactivation as determined by six-hour serum bioassay concentrations following a 4-mg. per kg. test dose (0.8+ = "slow"; 0.2 or less =

TABLE 2

INTERPRETATION OF SERUM ISONIAZID BIOASSAY RESULTS AS REPORTED IN THIS LABORATORY

(in γ/ml.)

Bioassay Report	Range	Mean
<0.2	0–0.1	0.05
0.2	0.2–0.3	0.25
0.4	0.4–0.7	0.55
0.8	0.8–1.5	1.15
1.6	1.6–3.1	2.35
3.2	3.2–6.3	4.75
6.4	6.4–12.7	7.15

TABLE 3

SERUM ISONIAZID BIOASSAY CONCENTRATIONS AT TWO VERSUS SIX HOURS BY REVERSAL OF INFECTIOUSNESS IN ORIGINAL DRUG-SUSCEPTIBLE CAVITARY PULMONARY TUBERCULOSIS

(A Retrospective Analysis of Two Series of Patients)

2-Hour Serum-Isoniazid Concentrations	High Isoniazid-PAS (30 Patients) Months to Conversion						Still Positive at 12 Months	High Isoniazid-PAS + Streptomycin per Day (60 Patients) Months to Conversion						Still Positive
	2	4	6	8	10	12		2	4	6	8	10	12	
<0.2–0.2	0	0	0	0	0	0	0	0	1	0	0	0	0	0
0.4	1	0	1	0	0	1	0	4	4	0	0	0	0	0
0.8	2	0	1	0	1	0	1	14	5	1	0	0	0	0
1.6	7	1	0	0	0	0	1	10	3	2	0	0	0	0
3.2	10	1	1	1	0	0	0	16	0	0	0	0	0	0
Totals...	20	2	3	1	1	1	2	44	13	3	0	0	0	0
6-Hour Serum-Isoniazid Concentrations	**High Isoniazid-PAS (30 patients) Months to Conversion**						**Still Positive at 12 Months**	**High Isoniazid-PAS + Streptomycin per Day (60 Patients) Months to Conversion**						**Still Positive**
	2	4	6	8	10	12		2	4	6	8	10	12	
<0.2–0.2	6	0	2	0	1	1	1	19	10	1	0	0	0	0
0.4	1	1	0	0	0	0	1	7	0	0	0	0	0	0
0.8	8	0	0	1	0	0	0	11	3	2	0	0	0	0
1.6	5	1	1	0	0	0	0	7	0	0	0	0	0	0
3.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Totals...	20	2	3	1	1	1	2	44	13	3	0	0	0	0

High = 12+ mg. of isoniazid per kg.

Streptomycin dosage adjusted by age, serum concentration, and renal function.

Conversion = culture only.

One case classed as "still positive" was a temporary conversion at three months with relapse at twelve.

"rapid"; 0.4 = "intermediate") has occasional relationship to results with isoniazid-containing regimens. Perhaps 5 or 10 per cent of "rapid" inactivators receiving isoniazid 300 mg.-PAS, isoniazid 300 mg.-streptomycin twice weekly, or isoniazid 300 mg.-PAS-streptomycin twice weekly will not do as well bacteriologically as comparable patients with advanced pulmonary tuberculosis manifesting "slow" inactivation.[2] This apparent difference seems to disappear when "high" isoniazid *plus streptomycin daily* with or without PAS is used. Some difference between "rapid" and "slow" inactivators who receive high isoniazid-PAS without streptomycin may emerge from larger and controlled studies.

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March 20, 1959

TUBERCULOSIS WAS FASHIONABLE IN 1759

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

In the present and past centuries tuberculosis was a scourge in Ireland and was known as the "white plague," the mortality rate being very high from this disease. Strange to relate, two centuries ago it was regarded as one of the elite diseases in Ireland—just the complaint of those of bluer blood—and it was called the "gentleman's decline." We know it was, strange to relate, the ill of the flesh of those who were well-fed as well as of those who were underfed down the ages; and high living too was a cause. The drinking of goat's whey or milk was made fashionable by the Irish nobility and gentry in the first half of the eighteenth century, and the cure of "TB" ranked as its chief medicinal property.

The obituary notice of an Irish squire in May, 1759, says: "He died of a speedy decline as is well becoming to a gentleman." Many young ladies in particular died from this complaint, the records reveal: "She was a young lady of fashion, most agreeable to all who knew her, a beauty unrivalled, who passed after a lingering illness in her chest."

It was an Irish doctor, Sir Henry MacCormac, M.D., who was early to proclaim that the disease was due to re-breathing air and that the best prophylactics were fresh air and ventilation. He got the sobriquet: "Fresh Air MacCormac." Certainly, the ill-ventilated rooms of country and town mansions with the well-draped tester beds were as ill an atmosphere as the thatched cabins of Ireland for the incubation of the dreaded tuberculosis.

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Ballynahinch
Co. Down, Ireland

March 20, 1959

[2] MITCHELL, R. S., AND BELL, J. C.: New England J. Med., 1957, *257*, 1066.

DIFFERENTIAL UPTAKE OF ISONIAZID-C^{14} BY MYCOBACTERIUM PARATUBERCULOSIS SUSCEPTIBLE AND RESISTANT TO ISONIAZID AND HYDROGEN PEROXIDE

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

In a previous work we reported our results which, in agreement with others, showed a relationship between the onset of isoniazid resistance and the disappearance of catalase activity by different strains of *Mycobacterium tuberculosis* and *paratuberculosis*.[1] This lack of catalase activity in isoniazid-resistant strains has been reported recently in connection with the increased susceptibility to hydrogen peroxide and the decreased virulence of isoniazid-resistant tubercle bacilli.[2] Other writers have established that there is a decreased isoniazid-C^{14} uptake in isoniazid-resistant tubercle bacilli.[3] However, Boone and associates reported no difference.[4]

We became interested in finding out if this decreased isoniazid-C^{14} uptake was specific for isoniazid-resistant strains or more generally for strains with diminished catalase activity, as for the strain resistant to hydrogen peroxide.

We employed four strains of *Mycobacterium paratuberculosis* with different susceptibility to isoniazid and hydrogen peroxide. The results obtained are reported herein.

Four strains of *Mycobacterium paratuberculosis* (M.PT.) (var. Minetti): normal (*SS*), isoniazid resistant (*RS*), hydrogen peroxide resistant (*SR*), and isoniazid and hydrogen peroxide resistant (*RR*), were used. The bacilli were cultured at 37°C. in Proskauer medium with Tween® 80 (1 per cent). The susceptibility test was carried out in test tubes containing 5 ml. of medium inocu-

[1] GARATTINI, S., *et al.*: Gior. ital. chemioterap., 1956, *3*, 85.

[2] COHN, M. L., *et al.*: Am. Rev. Tuberc., 1954, *70*, 641.

[3] BARCLAY, W. R., *et al.*: Am. Rev. Tuberc., 1953, *67*, 490.

[4] BOONE, I. U., *et al.*: Am. Rev. Tuberc., 1957, *76*, 568.

TABLE 1

Number of Experiments	Strains	Susceptibility to Isoniazid	Susceptibility to H_2O_2	Fixation of Isoniazid-C^{14} CPM*/Mg. Bacilli ± S.E.†
		γ/ml.	γ/ml.	
9	SS	1	30	135 ± 11
7	RS	50	20	60 ± 7
7	SR	20	80	45 ± 6
7	RR	20	>100	24 ± 1

* Counts per minute.
† Standard error.

lated with 0.01 mg. of cells (wet weight) per ml. The growth evaluation has been determined turbidimetrically by comparison with a series of standard dilutions. Isoniazid[5] was Seitz-filtered, added at the final concentration of 1, 2, 5, 10, 20, 40, and 60 γ per ml., and H_2O_2 was added at the approximate concentration of 5, 10, 20, 40 and 80 γ per ml.

The experimental conditions were as follows: A culture of M.PT. (var. Minetti) was inoculated (0.01 mg. per ml.) in a 500-ml. Erlenmeyer flask containing 200 ml. of medium; four hours after incubation 2 mc. of radioactive isoniazid,[6] dissolved in 1 ml. of distilled water, were added to each flask and the cultures were incubated for an additional twenty-four hours. The cultures were heated at 60°C. for one hour and the bacilli were centrifuged and washed repeatedly with saline. Aliquots of the bacillary suspensions were plated on weighed copper discs and counted with a gas-flow counter.[7]

After the usual corrections, the specific activity (counts per minute/milligram of dry bacilli) on each disc was calculated. The results obtained are reported in table 1.

The data reported confirm the results of Barclay and associates,[3] since in our experiments also a remarkable difference in the isoniazid-C^{14} uptake by the isoniazid-susceptible and isoniazid-resistant strains was found. However, the strain resistant to hydrogen peroxide also had a significantly lower uptake, and this may be only in connection with the increased resistance to isoniazid in this strain. In the case of the *RR* strain, although the resistance to isoniazid was the same as in the *SR* strain, with the increase of hydrogen peroxide resistance, there was a significant decrease of the isoniazid uptake. This seems to us to prove that a decreased isoniazid uptake cannot fully explain the mechanism of the resistance of the paratubercle bacilli to isoniazid, since other factors, like the resistance of H_2O_2, give the same effect.

Both strains *SR* and *RR* are catalase negative,[1] and this fact can suggest the importance of the catalase activity for the uptake of isoniazid.

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March 26, 1959

[5] Supplied by Maggioni, S. A. Milano.
[6] Isoniazid-carbonyl-C^{14} −2mC/mM obtained from The Radiochemical Centre, Amersham.
[7] SC −16 Tracerlab-Inc. gas-flow counter.

REHABILITATION OF TUBERCULOUS PATIENTS

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

You were kind to let us see the communication from Dr. Allen and allow us to comment on it.

We were gratified to learn of Dr. Allen's acceptance of the thesis that physical activity is not contraindicated in these days of potent chemotherapy of tuberculosis. However, while we agree with these concepts, we cannot accept Dr. Allen's limited approach to rehabilitation as expressed in his letter.

Perhaps Dr. Allen does not conceive of rehabilitation as we do, since he seems to refer only to the vocational aspects of adjustment. To our way of thinking, rehabilitation is an integral part of modern medical practice and includes all aspects of physical, emotional, social, and vocational adjustment. After reviewing his article "The Ex-Tuberculous Patient as An Employee" we think that perhaps he has not recognized all of the rehabilitation services that he has personally provided for his patients and that, generally, he may not be aware of all that is involved in rehabilitation. One can, of course, elect to do the total job himself, but we have found rehabilitation to be more effective when the services of specially trained individuals are utilized, such as social service, occupational therapy, and psychology.

We agree that occupational therapy, used solely as a means of preventing unsanctioned discharges, is a woefully incomplete and erroneous use of these services. Occupational therapy and other rehabilitation services should not be relegated to the position of fabricating "worthless items," but should be developed into a constructive program through which patients can be helped to maintain their basic skills and be motivated to acquire independence. Our experience clearly indicates that rehabilitated patients do not become "recipients of welfare" but go on to assume full and steady employment after discharge from the hospital.

Rehabilitation is based upon the knowledge

and confidence that patients of all backgrounds can in individual instances be helped to improve their general adjustment, even during brief periods of hospitalization. We know, however, that the staff must be convinced of the worthiness of the patient if rehabilitation is to be successful. Furthermore, we recognize that occupational therapy facilities alone do not influence the rate of unsanctioned discharges. Unsanctioned discharges relate to the degree of the patient's anxieties about his illness, hospitalization, and treatment. It is important to understand that the relationship between staff and patient is the essential element in meeting the problem of unsanctioned discharges and in carrying out a meaningful rehabilitation program.

Yes, we agree that many patients can and should return to their former occupations. But this should not preclude the opportunity of helping some patients achieve greater economic and emotional security through adding to their skills so that they can better compete in the employment market.

We note, too, that Dr. Allen has neglected to refer to those patients who fail to respond to initial chemotherapy and become persistent excreters of resistant organisms. This group ranges up to 10 per cent of the number of hospitalized patients, and in many cases these individuals need rehabilitation in order to maintain their self respect and independence. Apparently Dr. Allen must be in a particularly fortunate setting if, in addition to the latter cases, he is not faced with the problem of treating patients who have extensive disease with limited physical capacities so that they may not be able to return to their former occupations.

Again, with respect to Dr. Allen's paper, "The Ex-Tuberculosis Patient as an Employee,"[1] in which he sets forth his opinions about rehabilitation, we found it rather difficult to follow his statistical presentation. For example, he gives data on the present occupation of 300 patients, but at the same time states that, of a total of 350 admissions, 20 died during hospitalization, there was no follow up on 32 patients, and 8 per cent (presumably approximately 26 patients) left the hospital as irregular dischargees. On the basis of these figures, this leaves a balance of 272 patients. We wonder, therefore, how he presents statistics on the present occupation of a total of 300 patients.

In conclusion, let us consider a very important point. It seems obvious that the function of medicine is not to treat the organic disorder alone, but to provide assistance in all areas of adjustment that will help the individual return to society, accepting responsibility for himself and others who may be dependent upon him. One must realize that illness, acute and especially long-term, produces certain anxieties and problems which cannot be swept under the carpet by denying their existence. Rehabilitation viewed broadly can provide the necessary assistance in bridging the gap between illness and well being. It seems logical that the permitted increase in physical activity and the protracted and consistent chemotherapeutic regimens necessary for the inactivation of pulmonary tuberculosis have provided an unparalleled opportunity to establish total rehabilitation programs for tuberculous individuals.

Finally, the variables inherent in human biology demand careful evaluation of any generalized thesis that affects welfare of patients.

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April 7, 1959

[1] ALLEN, A. R.: Indust. Med., 1956, *25*, 573.

Books

TUMORS OF THE LUNGS AND MEDIASTINUM. By B. M. Fried. Pp. 467, Lea and Febiger, Philadelphia, 1958. Price $13.50.

This monograph is primarily devoted to carcinoma of the lung. A presentation of the multiple aspects of this disease is made in the first twelve chapters of the book and includes a consideration of the general principles of pulmonary function and radiotherapy. An outstanding section entitled "Surgical Treatment" has been written by Burford and Ferguson. They draw primarily on the wide experience at the Barnes Hospital which makes this chapter particularly valuable. The chapter on "Metastases" is well prepared and contains a great deal of helpful material. The author presents data devoted both to metastases *from* cancer of the lung and metastases *to* the lungs from extrapulmonary sources.

The mediastinum is less extensively covered and comprises approximately a fourth of the text. Representative cases of the various lesions are well chosen and the illustrations of the chest films, gross specimens, and photomicrographs are excellent. The bibliography for the mediastinal lesions is extensive and carefully selected and provides a valuable reference source.

The book may be recommended to those interested in the diagnosis, management, and teaching of tumors of the lungs and mediastinum. It is a concise and easily readable source of the most recent views on this important aspect of chest disease.

DAVID C. SABISTON, JR.
Baltimore, Maryland

JOHN JACOB ABEL, M.D. A Collection of Papers by and about Him. Pp. 80, The Williams and Wilkins Company, Baltimore, Maryland, 1957.

This collection of papers was assembled and published by the Williams and Wilkins Company, Baltimore publishers, as a memorial to Abel on the hundredth anniversary of his birth. The Williams and Wilkins Company is particularly indebted to Abel because it was he who persuaded the company to undertake publication of the *Journal of Pharmacology and Experimental Therapeutics*. A series of other scientific journals and books followed.

The collection is made up of two biographical sketches by Abel's former students, E. K. Marshall, Jr., and Paul D. Lawson, and four important papers by Abel and his colleagues: "On the Blood Pressure Raising Constituent of the Suprarenal Capsule," "On the Teaching of Pharmacology, Materia Medica and Therapeutics in Our Medical School," "On the Removal of Diffusible Substances from the Circulating Blood by Means of Dialysis," and "Crystalline Insulin." These papers were originally published in the *Bulletin of the Johns Hopkins Hospital*, the *Transactions of the Association of American Physicians*, Abel's own *Journal of Pharmacology and Experimental Therapeutics*, and the *Proceedings of the National Academy of Sciences*.

The result of this small book is the creation of a convincing picture of the "investigator, teacher, prophet" who isolated epinephrine, invented the "artificial kidney," prepared the way for use of the phthaleins in the clinical study of renal and biliary function, and crystallized insulin. Abel's approach to pharmacology was strongly chemical in its orientation. He had a powerful influence on American medicine and science as a result of his long period as Professor of Pharmacology and Experimental Therapeutics at Johns Hopkins, as founder and editor of the *Journal of Pharmacology and Experimental Therapeutics*, as joint Editor of the *Journal of Biological Chemistry*, and as a founding father of a least two scientific societies.

VICTOR A. MCKUSICK
Baltimore, Maryland

STAPHYLOCOCCAL INFECTIONS. By Ian Maclean Smith. Pp. 180, The Year Book Publishers, Inc., Chicago, 1958. Price, $4.25.

Dr. Smith has attempted in this volume to summarize available knowledge on the subject of staphylococcal infections. He states in the preface that the volume is primarily for the use of the general practitioner. In orienting the manual in this fashion, Dr. Smith has chosen to present a panorama of the clinical manifestations of staphylococcal infections in both man and animals. He has also included a brief section on laboratory characteristics of staphylococci. The volume concludes with a summary of present day therapy in regard to both specific antimicrobial and nonspecific supportive regimens.

The manual may well be of considerable value for both practicing physicians and medical students. It has, however, some distressing shortcomings. First, despite the clinical orientation of

the volume, the author fails to utilize adequately case histories, roentgenograms, or illustrations to clarify and emphasize points made in the manuscript. Second, the panoramic view inevitably tends to be superficial throughout so that the reader is almost invariably left with insufficient knowledge of any given subject. Third, the author does not provide references for his statements or document them with examinable evidence. Many of the statements on incidence or prevalence, clinical manifestations, and therapy are at variance with other published data or represent one view of unsettled issues. It might be considered unwise to ask the reader who is unschooled in the staphylococcal literature to accept such statements as a basis for important clinical decisions.

DONALD B. LOURIA
New York, New York

BOOKS RECEIVED

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American Trudeau Society

Medical Section of the National Tuberculosis Association

The Chest Roentgenogram and Chest Roentgenographic Surveys Related to X-Ray Radiation Effects and Protection from Radiation Exposure

A Statement of the Committee on Radiation Effects

This report is a revision of an earlier statement of the American Trudeau Society issued in October, 1957.

The roentgenographic examination of the lungs has been one of the most important and dependable tools of the thoracic physician and surgeon and public health worker in early diagnosis and treatment of unsuspected chest disease. The chest roentgenogram continues to be an important part of all tuberculosis case-finding programs. The National Tuberculosis Association in 1955 summarized the modern usefulness of the technique of chest roentgenographic surveys with far-reaching principles concerning its application. The following are a few of the recommendations it stressed:

> Tuberculosis, lung cancer, nontuberculous pulmonary infections, intrathoracic tumors, and cardiovascular abnormalities are conditions whose lasting cure often depends upon early diagnosis of the unsuspected lesion by chest X-ray techniques.
>
> Immediate follow-up and proper handling of every significant thoracic abnormality is necessary.
>
> The general hospital roentgenographic admission program as a survey technique is a most productive method for turning up previously unsuspected thoracic disease.

In June, 1956, the National Academy of Science, National Research Council, called attention to the effects of ionizing radiation on the human body and its reproductive organs. Many aspects of this subject remain controversial, but this discussion on radiation effects has led everyone—scientist, physician, and layman—to think deeply concerning them and to evaluate programs utilizing ionizing radiation.

The problem is therefore one of weighing the benefits from roentgenographic diagnostic procedures against the possibility of harmful effects from radiation exposure.

There are few good human data on this aspect of low doses of ionizing radiation, and most of the factual information has come from animal experimentation. Nonetheless, the unknown as well as the known effects of radiation exposure impose a definite responsibility to the public.

In people who are ill, the needs for roentgenographic studies are great and deserve more weight on the side of diagnostic benefits as opposed to possible hazardous effects of radiation. In any case, all radiation exposure that serves no useful purpose should be scrupulously avoided.

It is well recognized concerning X-ray machines that there is no standard pattern of radiation exposure delivered by any standard type of unit. It has been shown that each X-ray unit, photofluorographic or conventional, delivers its own peculiar amount and type of X-ray radiation. Therefore, each machine must be tested individually and carefully for its own output of radiation and must be provided with all necessary safety devices for minimizing gonadal and general body radiation. This must be done by persons trained in radiologic protection.

I. Who should get roentgenograms: The American Trudeau Society has taken leadership in emphasizing that there is no virtue in the taking of chest roentgenograms per se. Chest roentgenograms for case finding are justified only if they lead to the detection of previously unsuspected or clinically significant lung disease, followed through with appropriate therapy. If the abnormalities disclosed are not followed up, radiation has been needlessly used.

Therefore, it is essential for those engaged in the detection of pulmonary disease to evaluate their yields on a continuing basis. These yields vary from one community to another and from time to time but, in general, are higher among certain population segments. Among these high-yield groups, periodic chest roentgenograms are the most practical approach. Among infants, children, young adults, and prenatal patients, the tuberculin test should be used as the preliminary screening technique whenever possible, and the tuberculin reactors should have roentgenographic examinations of the lungs for the detection of tuberculosis. The nonreactors among these groups should be rechecked periodically by means of the tuberculin test. In children a single routine chest roentgenogram may be justified for the identification of unsuspected congenital or developmental defects and nontuberculous disease, and for comparison with any films taken later in life. Only those X-ray units that meet modern requirements for radiation protection should be used.

II. What type of apparatus should be used?: Three types of equipment are in general use for chest roentgenographic survey purposes. Other factors being equal, the amount of radiation necessary for a satisfactory chest film is least with a standard 14- by 17-inch film in a cassette with intensifying screens. Compared with the standard 14- by 17-inch X-ray unit, there is approximately three to five times more radiation using the mirror optics photofluoroscopic unit and about ten to twenty times the radiation exposure when using the standard lens camera photofluorographic machine. This in well-protected X-ray units is still a very small amount of radiation, but these figures may be multiplied by 100 if the apparatus is not properly equipped with protective devices. This difference in radiation exposure between the standard 14- by 17-inch and either photofluorographic X-ray unit may be less than the difference between a well-protected and a poorly protected machine.

When the number of survey films taken per day is small it is better to use standard 14- by 17-inch films. When the number of films taken per day is large, or the machine must be moved frequently as in the case of a mobile unit, a properly equipped photofluorographic unit is the most practical apparatus at the time, and its use is indicated in those segments of the population in which the yield of new cases is significant.

Whenever the purchase of a new photofluorographic unit is contemplated, the mirror optical system camera is to be preferred over the ordinary lens system. The reduction in radiation with this device and the superiority of results will justify a substantial difference in price.

Screening of groups by fluoroscopy should be strongly discouraged for several reasons: the results are not accurate for diagnostic purposes; there is no permanent film record of the examination; and the radiation exposure involved both for the subject and examiner is excessive. However, special fluoroscopic examination may be indicated for specific diagnostic purposes and for the determination of the dynamics of the chest.

Body-section roentgenograms even with properly operated equipment can result in a substantial radiation exposure to the chest, and should be used only for special diagnostic problems.

III. The nature of radiation effects: Populations are being exposed to a variety of radiations from natural and artificial backgrounds as well as from medical diagnostic and therapeutic procedures. The average exposure received by the population today from all sources appears to be at a lower level than that which has been demonstrated to produce harmful effects in humans and experimental animals.

At this time the effects of small doses of radiation are unknown. Speculation is based on statistical calculations projected downward from the known effects of higher doses. These effects from higher doses may manifest themselves as genetic changes resulting from radiation of the gonads, or general body effects with leukemia or other delayed pathologic changes as rare sequelae. It should be emphasized that unnecessary radiation should always be avoided by conducting medically supervised programs with properly operated equipment and adequate follow-up plans.

IV. Techniques for minimizing exposure: Those responsible for screening programs should ensure that steps have been taken to maintain the radiation dose at the lowest practicable level both to those being examined and to equipment operators. Techniques are available to accomplish this at nominal cost, and should be applied wherever not already in use. These techniques include:

1. *Proper coning.* This is particularly important in reducing the gonadal dosage. Cones should be rectangular in shape and should limit the primary beam to an area no larger than that visualized on the film. Adequate cones should be continuously mounted on all units. Misalignment of the cone results in "cone cutting" of film. Technicians often remove cones or use too large an aperture to avoid "cutting" the film. This should never be permitted. Precise and accurate alignment, rather than removal of the cone, is the proper remedial procedure in this case. Mechanical linkage of the tube and film holder or the use of light localizers are recommended.

2. *Proper filtration.* This should be assured by permanently mounting at least 2 mm. of aluminum filtration, or its equivalent, in the primary beam. When technical factors permit, filtration up to 3 mm. should be used, and this is better than 2 or 2.5 mm.

3. *Shielding.* Proper shielding of both the direct beam and the scatter radiation should be provided. Its adequacy for protection of equipment operators, their assisting personnel, waiting patients, and the general public should be assured by adequate radiation surveys.

4. *Adequacy of tube housing.* This is a protective barrier and should be checked by a radiation survey. In addition to its over-all effectiveness, special attention should be paid to the possibility of small defects which will result in leakage of direct radiation in directions other than that of the useful beam.

5. *Technical factors.* Significant reductions in patient and operator dosage can often be obtained by use of higher kilovoltage techniques. Optimal operating voltage will vary with equipment. Higher speed screens and films should also be employed.

6. *Tube-to-skin distance.* This should be kept as great as possible. Five- or six-foot distances are desirable.

7. *Exposure control.* These should be so arranged that operation of the equipment is impossible except with the operator behind a suitable protective barrier.

8. *Radiation monitoring.* There is no real substitute for a thorough radiation safety survey of X-ray equipment by a properly qualified individual, repeated at regular intervals, preferably once a year. Such monitoring should be the goal of all users of X-ray equipment. Official health agencies, radiologists, and radiation physicists can often supply properly qualified and equipped persons to perform necessary services.

In addition, more advanced and expensive technical improvements can result in a reduction of patient and operator dosage. Examples of these improvements are mirror optics and electronic image amplification. Widespread applications of such techniques can be deferred, however, pending a concerted effort to accomplish the modifications noted above, when needed. Early implementation of recommendations regarding coning and filtration should be undertaken without awaiting availability of complete radiation surveys.

Where not already accomplished, suitable instruction of personnel in optimal operating and film processing techniques should help keep diagnostic value high and minimize the necessity for re-examinations.

The principles outlined above are equally applicable in individual diagnostic studies. When fluoroscopy is involved, additional emphasis should also be placed on minimal field size, adequate adaptation to darkness, and tube-to-patient distance which should be at least 18 inches as well as an automatic timer for power cut off.

Conclusions: The kernel of the problem of radiation effects is the awareness by the public, physicians, and tuberculosis workers that the whole subject is one of weighing the benefits of radiography against the known and possible effects of radiation exposure. It should remain clear that radiation that serves a useful and necessary purpose is warranted, but it should be used with the best protective devices to minimize exposure. Putting the chest roentgenographic examination in its proper perspective, when a properly monitored machine is used, the effects of radiation exposure to the gonads or body from a single chest film for each individual are small when the benefits derived from a carefully controlled and properly executed case-finding program are considered.

Recommendations: Several specific recommendations from this report can be made to the constituent associations of the National Tuberculosis Association and American and state Trudeau Societies.

1. Chest roentgenographic surveys must be continued for the detection of tuberculosis, cancer, industrial thoracic disease, acute and chronic nontuberculous infections, chest tumors, and cardiovascular abnormalities.

2. Conventional and photofluorographic X-ray units may be used to survey segments of the population which are expected to show a high yield of thoracic disease, but the X-ray machines must be equipped with adequate protective devices.

3. Tuberculin testing in infants, children, young adults, and prenatal patients should be developed as a primary guide to tuberculosis contacts. A roentgenogram of the chest for the detection of tuberculosis should then be limited to those with a positive tuberculin test in these groups.

4. Reassessment of case-finding program should be done to determine those segments of the population which should be examined roentgenographically and those which should be tuberculin tested.

5. The instruction and training of personnel should include information concerning the protective devices for all types of X-ray units. Members of the American Trudeau Society and constituent associations of the National Tuberculosis Association should assume an active role in assisting in and promoting the training of personnel skilled in radiation protection.

6. A constructive approach is in order to emphasize the continuing usefulness and the need for early diagnosis and treatment of all forms of pulmonary disease.

Richard H. Chamberlain
E. Osborne Coates, Jr.
John H. McClement
Clinton Powell
Joseph B. Stocklen, *Chairman*

"The Open-Negative" Problem

A Statement of the Committee on Therapy

The "open-negative" problem is largely a product of the chemotherapy era. Formerly the persistence of open cavity on the roentgenograms of a tuberculous patient was almost invariably associated with the ability to recover tubercle bacilli from his secretions. Today, however, the majority of patients receiving a first course of effective chemotherapy for cavitary tuberculosis pass through a phase of bacteriologic remission while one or more cavities are still open. In some, this is followed by cavity filling or cavity closure leading to an inactive classification. In others, the bacteriologic remission and persistent open lesion coexist for months or even years. This is the group of cases that constitute the open-negative problem.

The problem consists of the fact that, after eight years of experience with this phenomenon, agreement on the treatment and prognosis of these patients is lacking. It is the committee's belief that much of the conflict and confusion surrounding this problem is the result of the different ways in which various reporters use the terms "open-negative" and "relapse." The committee, therefore, submits the following thoughts and recommendations in the hope that some of this confusion and conflict might be dispelled.

Definition: For the purposes of this report an "open" lesion is a hole or space in an area of the lung previously involved by tuberculosis, containing air and usually surrounded by a wall of sufficient density to be recognizable as such morphologically or roentgenographically. Not included are: (*1*) areas of increased radiolucency of segmental or lobar extent without a definite wall; and (*2*) former cavities or spaces, whatever their original nature, from which all air has been absorbed or extruded; and (*3*) bullae, cysts, and areas of cystic or saccular bronchial dilatation when these can be differentiated with reasonable certainty from residual tuberculous cavities. It should be recognized that this latter distinction is not always possible even with the most careful and detailed roentgenographic techniques.

The duration of bacteriologic remission required for inclusion in the open-negative category must be arbitrary. It is recommended that tubercle bacilli shall have been absent from the sputum and gastric contents examined at monthly intervals for at least six months. It is further recommended that, in reporting experience and results, the designation "open-negative" be supplemented by indicating the duration of bacteriologic remission: thus "open-negative 6 months" or "open-negative 18 months." (Comment: In this way correlations may be drawn between duration of bacteriologic remission and prognosis. If, for example, the relapse incidence is reported for a group of patients who had remained open-negative for an *average* of twelve or eighteen months, the picture is incomplete unless one knows how many patients open-negative for three, six, or nine months were removed from the group because of bacteriologic relapse short of the twelfth or eighteenth month.)

Incidence and diagnosis: It has been estimated that approximately 90 per cent of patients who receive a first course of effective chemotherapy for cavitary pulmonary tuberculosis will achieve bacteriologic remission (reversal of infectiousness, sputum conversion) during the first eight months of treatment. More than half of such patients, however, will still have roentgenographic evidence of cavity if examined carefully at the end of this eight-month treatment period. This incidence has been reported independently by several groups, but may be influenced somewhat by the type of cases being observed and by the care and thoroughness with which the search for residual open lesions is made.

The diagnosis of an open lesion on chest roentgenograms or planigrams preoperatively is followed by the discovery of an open lesion in the resected specimen in the majority of cases. Careful review of serial roentgenograms is much more reliable in predicting the presence of an open lesion than the polling of several observers, however expert, in an attempt to make this determination in a single film or a single set of planigrams. Bronchograms may help to distinguish one type of open lesion from another, but cannot do so with finality. Even gross examination of the resected specimen itself may leave the exact nature of the open lesion in doubt. In general, however, an open lesion of some kind will be found in the resected specimen in 75 to 85 per cent of cases when an open lesion is reasonably suspected after careful examination of serial roentgenograms. The more equivocal the open lesion is on roentgenograms, the poorer this correlation becomes.

Pathology and bacteriology: Although much has been made of the advanced state of healing in some resected "open-negative" lesions, this is a relative thing which is usually compared with the morphologic appearance of cavities resected without drug coverage or obtained at autopsy. Nevertheless, even today with effective chemotherapy, the majority of resected "open-negative" lesions

show residual caseous foci and granulation tissue irregularly distributed in and along the cleanly sloughed, fibrotic cavity wall.

It has been shown that the longer the period of bacteriologic remission prior to resection in surgically treated cases, the more advanced the healing process appears morphologically and the more difficult it is to recover viable bacilli from the resected specimen. A similar correlation between the duration of bacteriologic remission and the morphology and tissue bacteriology in unresected open-negative cases is often assumed, but the fact that it is an assumption lacking specific proof should be borne in mind.

Relapse: If one wishes to measure the prognostic significance of the "open-negative" state by the incidence of relapse, one must arbitrarily define relapse. Simultaneous roentgenographic worsening and positive bacteriologic findings are universally accepted as evidence of relapse. The insertion of provisions such as "relapse requiring hospitalization" or "relapse requiring further treatment" introduce subjective factors originating in both the patient and his physician and, while they may indicate the seriousness or significance of a relapse, should not in our opinion be used in measuring its *incidence*. It is proposed that recovery of tubercle bacilli by any method—sputum culture or smear or gastric culture—occurring in a patient classified as "open-negative" be considered a relapse. While it might seem desirable to require more than a single positive bacteriologic finding for relapse, the tendency to introduce new drugs or other therapeutic measures after even a single culture positive for tubercle bacilli, especially when unsuspected drug resistance is noted, is very common and a second positive bacteriologic finding might thereby be prevented or masked. This would tend to lower the relapse incidence artificially.

Unfavorable roentgenographic changes with smears and cultures repeatedly negative for tubercle bacilli may be due to nontuberculous pulmonary disease. Such changes, therefore, should rarely, if at all, be counted in tabulating tuberculosis relapse incidence in the open-negative group of cases. The physician of such a patient may elect quite properly to treat him as a case of tuberculous relapse in the absence of bacteriologic confirmation, but for statistical purposes such cases should be classified separately, not as relapses. The same applies to patients who show signs of clinical worsening such as fever, weight loss, increased cough, expectoration, or even hemoptysis without roentgenographic change or bacteriologic confirmation of tuberculous etiology.

The role of indefinite prolongation of drug therapy in the prevention of relapse needs investigation. The earlier a relapse occurs, the more likely it is to occur while drugs are still being given; the later the relapse occurs, the more likely it is to follow cessation of chemotherapy. Thus, those who require nine to twelve months of bacteriologic remission for inclusion in the "open-negative" category are more prone to see relapse after therapy has stopped. The earlier bacteriologic escapes, during the sixth to twelfth months of therapy, have been dropped by definition. Conversely, those who require shorter periods of bacteriologic remission for designation as "open-negative" tend to see relapses more frequently while therapy is still being given. This does not imply that some of the post-therapy relapses in both groups might not have been prevented by further prolongation of treatment.

In summary: The problem of persistent open cavity with bacteriologic remission of greater or less duration is a phenomenon peculiar to the era of effective chemotherapy for tuberculosis. The roentgenographic findings fairly represent the morphologic findings in the majority of patients whose lesions are resected and may also reflect the findings in those lesions which have not been resected.

The definition of "open-negative" based on the presence of a cavity and inability to recover tubercle bacilli from secretions is arbitrary, but a bacteriologic remission of six months or longer is recommended for inclusion in this category.

Relapse should be defined as either bacteriologic escape or combined roentgenographic-bacteriologic worsening, but not as clinical or roentgenographic worsening without bacteriologic proof of its tuberculous etiology.

The relationship between relapse, duration of bacteriologic remission, and the duration of effective drug therapy is in need of further study. Not until this has been done can the relative risk of relapse and the risk of resection in individual patients exemplifying the "open-negative" problem be fairly weighed and balanced.

Thomas B. Barnett
Edward Dunner
H. Corwin Hinshaw
Gardner Middlebrook
Donald L. Paulson
James W. Raleigh
William W. Stead
James A. Wier, *Chairman*

Obituaries

Lyman I. Thayer

1893–1957

Dr. Thayer was born in New Jersey, and was educated at Colby College in Maine and at the College of Physicians and Surgeons of Columbia University, in New York. Following an internship in the Albany Hospital he entered the service of the New York State Department of Health and was assistant director of its Tuberculosis Division from 1921 to 1929. He then became Superintendent of the Westmount Sanatorium in Glens Falls, New York, a position which he held for twenty-four years. During the last four years of his life he was assistant director of the State Tuberculosis Sanatorium in Paris, Kentucky.

While living in Glens Falls, Dr. Thayer participated in many of the social, fraternal, civic, religious, and professional activities of the community. He was also a member of the American Trudeau Society and of the American College of Chest Physicians. For several years he was a member of the Board of Visitors of the New York State Tuberculosis Hospital at Ray Brook. He died of a heart attack on November 9, 1957, while undergoing diagnostic studies at the Mayo Clinic. He is survived by his widow, Mrs. Ruth M. Thayer

R.C.M.

Harold H. Brueckner

1907–1957

Dr. Brueckner was graduated from the Medical School of the University of Michigan in 1932. Following an internship he served on the resident staffs of the Irene Byron Sanatorium in Fort Wayne, Indiana; the State Sanatorium in Howell, Michigan; and the Florida State Sanatorium in Orlando. In 1938 he joined the staff of the Waverly Hills Sanatorium in Kentucky and was appointed clinical instructor in the nearby Medical School of the University of Louisville. In 1941 he resigned from this position and became Medical Director of the District Tuberculosis Sanatorium in Lima, Ohio. Five years later he went from Lima to Canton, Ohio, where he was Superintendent and Medical Director of the Molly Stark Sanatorium from 1946 until shortly before his death. He died of polycystic kidney disease on December 8, 1957.

Dr. Brueckner was a member of the American Trudeau Society for eighteen years and of the American College of Chest Physicians. He was active in the affairs of the Ohio Trudeau Society, and was well known and highly respected by his colleagues in tuberculosis work throughout the state. As Director of the Sanatorium, Dr. Brueckner was an effective member of the team of anti-tuberculosis workers whose efforts gave Stark County in recent years the proud record of having the lowest tuberculosis death rate of any industrialized community in Ohio.

R.C.M.

Arthur W. Dahlstrom

1890–1958

Dr. Arthur W. Dahlstrom of Albuquerque, New Mexico, died on August 26, 1958, as a result of bilateral subdural hematoma and arteriosclerosis. He obtained his medical degree from the Marquette University School of Medicine in 1916. In 1935, Dr. Dahlstrom entered the Medical Division of The United States Indian Service where his interest in tuberculosis was immediately manifested. In 1937–1938 he took special training in tuberculosis and public health at the Henry Phipps Institute. Following that period he was appointed Tuberculosis Control Officer for The United States Indian Service and embarked upon a program which has been of tremendous benefit to our Indian population and to American medicine. He first made a complete study of the tuberculosis problem on every Indian reservation. This study included consideration of the health, social, and economic conditions of nearby towns and villages.

After World War II he set up two mobile X-ray units which visited every Indian center in the United States. Ninety per cent of the Indian population participated in this survey. When the extent of the problem was determined, Dr. Dahlstrom instituted a BCG program, setting up clinics at missions and boarding schools on each reservation. Infants up to six months were vaccinated, using as controls all children born in presidential election years. Before his retirement in 1955, Dr. Dahlstrom had initiated the BCG vaccination at the hospitals so that all Indian infants were vaccinated within forty-eight hours of birth. Since 90 per cent of Indian births now occur in hospitals, the importance of this program is apparent.

After retiring in 1955, Dr. Dahlstrom became affiliated with the University of Pittsburgh in a field study dealing with the use of isoniazid in the Pueblo and Ute Indians.

Dr. Dahlstrom's humane concern for his Indian charges rivalled his medical zeal. As a result of his efforts, tuberculous meningitis has been nearly eliminated as a cause of death among Indian children.

He will be sorely missed in the hogans, the tepees, and Indian lodges throughout the West.

J.D.M.

Mack McConkey

1893–1958

Dr. Mack McConkey, a native of Oklahoma, began his professional life as a pharmacist. During World War I he served for two years in the U. S. Army Medical Corps as a Second Lieutenant. He was later graduated from Mt. Union College and from the Harvard Medical School. He served an internship in the Cleveland City Hospital. In 1926 he became a member of the medical staff of the New York State Hospital at Ray Brook and was assistant medical director of that institution from 1932 until his retirement in 1948. He was a member of the American Trudeau Society and the American Clinical and Climatological Association. For many years he served on the faculty of the Trudeau School.

Dr. McConkey was one of that large group of American physicians who have distinguished themselves by a lifetime of devoted professional service to their fellow victims of tuberculosis—the disease to which he eventually succumbed on April 14, 1958. Although his career was repeatedly interrupted by the necessity of "taking the cure," his extraordinary courage and equanimity were a source of inspiration to his patients and colleagues. Notwithstanding his physical limitations, he successfully kept alive a healthy spirit of scientific inquiry and was among the first to draw attention to the clinical significance of endobronchial tuberculosis and to the value of high vitamin therapy in the treatment of intestinal tuberculosis.

Dr. McConkey is survived by his widow, Mrs. Ellenor McConkey, who resides in Saranac Lake.

R.C.M.

NECROLOGY

Joseph D. Aronson, Philadelphia, Pennsylvania
Leonidas Forister Barrier, Little Rock, Arkansas
Richard C. Benkendorf, Bushnell, Illinois
William McBroom Blair, Wharton, Texas
Baldwin Borreson, Breckenridge, Minnesota
Edwin Herms Brady, Auburn, California
Johan Fredrik Bratt, Trondheim, Norway
Harold Hugo Brueckner, Canton, Ohio
Warren A. Brooks, Winter Park, Florida
Daniel M. Brumfiel, Saranac Lake, New York
Grover Stanislaus Brozozowski, San Diego, California
Samuel S. Cooley, Asheville, North Carolina
Arthur W. Dahlstrom, Albuquerque, New Mexico
Miguel Rivera Diaz, Quezaltenango, Guatemala
John A. Foley, Dorchester, Massachusetts
Morris Frishman, Pittsburgh, Pennsylvania
Edmund Gellert, Kerhonkson, New York
Frederick James Gray, Jr., Little Rock, Arkansas
George Emerson Gwinn, Beckley, West Virginia
Clara Jacobson, Chicago, Illinois
Herbert Bradley Kennedy, Omaha, Nebraska
William Ernest Kramer, West Monroe, Louisiana
Horst Lange, Seaford, New York
John H. Laurie, Montreal, Canada
Solomon S. Lubin, New York, New York
C. Howard Marcy, Pittsburgh, Pennsylvania
Edward August Meyerding, St. Paul, Minnesota
Janina Misiewicz, Warsaw, Poland
Mack McConkey, Saranac Lake, New York
Bradford H. Peirce, Hanson, Massachusetts
John Philip Schantz, Schuykill Haven, Pennsylvania
Frank G. Seldon, Manchester, New Hampshire
Max Shevell, Otisville, New York
Elihu F. Sloan, Columbiana, Alabama
Thomas Joseph Snodgrass, Janesville, Wisconsin
David J. Sobin, West Covina, California
Lyman Irving Thayer, Paris, Kentucky
Winston Harris Tucker, Evanston, Illinois
William Dunn Walker, Charlottesville, Virginia
Harry J. Wittenberg, Cincinnati, Ohio
Lawrence E. Wood, Kansas City, Missouri

NOTICES

The following dates and locations of the next three annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1960—Los Angeles, California—May 15-20
1961—Cincinnati, Ohio—May 21-26
1962—Miami Beach, Florida—May 20-25

FOURTH INTERNATIONAL COURSE ON TOMOGRAPHY

The Fourth International Course on Tomography will be held at the Radiology Institute of the University of Genoa, Italy, from September 28 to October 3, 1959, under the direction of Professor Alessandro Vallebona.

Information may be obtained from the general secretary of the course, Professor Neopolo Macarini, Istituto di Radiologia, Ospedale S. Martino, Genoa, Italy.

PAN AMERICAN MEDICAL ASSOCIATION CONGRESS

The Pan American Medical Association will meet in Mexico City from May 2 to 11, 1960.

For information write Dr. Joseph J. Eller, Director General, 745 Fifth Avenue, New York, New York.

SIXTH INTERNATIONAL CONGRESS FOR INTERNAL MEDICINE

The Sixth International Congress for Internal Medicine will be held in Basel, Switzerland, from August 24 to 27, 1960.

For further details, apply to the Secretariat of the Sixth International Congress for Internal Medicine, 13, Steinentorstr, Basel, Switzerland.

CONVENTION ON EPIDEMIOLOGICAL PROBLEMS OF TUBERCULOSIS

The Israel Association of Chest Physicians is organizing a Convention on Epidemiological Problems of Tuberculosis. This meeting will take place in Jerusalem, Israel, on September 22 and 23, 1959, after the XVth International Tuberculosis Conference in Constantinople.

Applications for registration for attendance should be sent to the Israel Association of Chest Physicians, P.O.B. 8123, Jaffa.